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(54) Imaging agent comprising a labelled malarial peptide

(57) Compositions of matter having affinity for erythrocytes and capable of binding to a Red Cell a labelling moiety; Such compositions being useful for diagnostics imaging as blood pool imaging compositions, comprise an agent of formula:

$(R^*_n - C_z) - L_m - P_x$

wherein

P is a peptide from a malarial parasite, or a deletion, or insertion, variant thereof;

L is an amino acid, polyamine acid of formula $(Xaa)_m$ or $(XaaZaa)_m$, or a non-malarial peptide fragment, as linker;

C is a chelator capable of binding to the side-chain of an amino acid and to a labelling entity;

R* is a labelling entity (particularly a radioactive, or luminescent, atom);

n,m,x and z are integers.

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The Field of the Invention:

The present disclosure relates to imaging agents useful for imaging blood vessels and their function i.e. Blood Pool imaging agents. The disclosure provides agents capable of imaging by means of contrast enhancement with X-rays or MRI and also labelled agents useful for imaging with gamma cameras.

These agents may be useful in cardiac or hepatic imaging or in vascular imaging generally.

Background Art:

Blood pool imaging agents (BPA's) are a group of diverse pharmaceuticals in the field of Radiology and Nuclear medicine whose purpose it is to assist the radiologist in producing pure vessel images. The production of a pure vessel image is helpful in diagnosing a wide range of pathology, from liver tumours, liver haemangiomas, to pulmonary or coronary infarcts, to blood flow before and after vascular surgery and many other applications.

Three broad groups of agent exist:

i. X-ray contrast agents - eg. iodine or halogen based agents;
ii. Radiopharmaceuticals - designed to produce an image using a gamma camera ie. radiolabelled entities;
iii. NMR nuclear magnetic resonance based contrast agents;

Until recently X-rays and CT (computerised tomography) scanning were the ultimate in image technology. Vascular contrast is developed using water soluble halogenated agents. Such agents possess the major disadvantage of leakage or diffusion from the intravascular space to achieve equilibration with the extra vascular space - 4 to 5 times larger. The effect of this diffusion is to

- lower the vascular concentration of agent;
- lower the contrast between vessel and surrounding tissue;
- a reduction of the available scan time.

Other disadvantag s with halogenated contrast media are nephrotoxicity and allergic reactions. The use of halogenated contrast media for coronary angiography is associated with a significant mortality 0.1%-0.3%. For these reasons MRI coronary angiography has been developed to lower the risks (see later). Nevertheless newer halogenated agents offer some improvements over older agents.

An example of such an agent is 'perfluorocytylbromide' PFOB Alliance Pharm. Corp San Diego CA, a new agent which may have the mentioned disadvantages to a lesser degree. Such agents are particularly helpful in distinguishing hepatic haemangiomas from a secondary hepatic deposit, a need brought about by the increasing feasibility of partial liver resections. At the present time it is difficult to make this distinction using MRI.

The problem of leakage of halogenated agents from the vascular space is the indirect cause of much of the morbidity and mortality associated with their use for the reasons that the contrast must be administered intra-arterially.

Intra arterial contrast administration involves the risk of damage to the vessel wall, dislodgement of emboli and in the case of coronary arteries the bolus effect may lead to arrhythmia or infarction.

To avoid arterial administration of contrast the problem of leakage must be addressed, thereby permitting the contrast to be administered intravenously.

One possible solution to this problem was disclosed by Doucet C et al "Blood-Pool X-ray Contrast Agents" Investigative Radiology November 1991 Supplement Vol 26, 553-54; who proposes iodinated Dextran polymer (Dextran 17,000). Administered intravenously to rats this polymer maintained higher intravascular plasma levels than those maintained by conventional halogenated agents. Revel D et al "Iodinated Polymer as Blood-Pool Contrast Agent - Computerised Tomography Evaluation in Rabbits" Investigative Radiology November Supplement 1991: Vol 26: 557-59: evaluated the same iodinated dextran as an agent for CT contrast and demonstrated substantial improvements over conventional agents, primarily caused by the slower diffusion of the polym r from the vascular space.

The halogenated agents of the present disclosure aim to solve the problem of contrast agent diffusion from the vascular space not by virtue of their molecular size but by their affinity for intra vascular cells.

Further the paramagnetic agents of the present disclosure may be used in place of halogenated agents provided for CT scanning provided a higher voltage is used, than that normally used with halogen compounds, 120-150 KeV.

One is directed to Zwicker C et al "Comparison of Iodinated and Non-iodinated Contrast Media in Computerised Tomography". Investigative Radiology November Supplement 1991 vol 26 5162-164 who discusses the ideal voltage settings (KeV) when employing paramagnetic elements Dy;GD;Yt as CT contrast agents.

The field of nuclear medicine and radionucleotide imaging began with the invention of the gamma camera and development of technetium^{99}mm in the 1960's. Radionucleotide imaging provides largely 'physiological' information as distinct from 'anatomical;' information provided by CT and MRI techniques.

Coronary angiographic imaging is increasingly called for to evaluate the efficacy of thrombolytic therapy, coronary angioplasty and grafting. The agent technetium^{99}mm labelled methoxyisobutylisonitrite MIBI represents an advance over thalium^{201} owing to the greater photon flux of technetium^{99}mm. However, this agent became fixed to myocardial cells necessitating several injections during exercise testing. Najin et al 1989 "The Evaluation of Ventricular Function Using Grated Myocardial Imaging with Tc^{99}mm" European Heart Journal 10, 142-148. Therefore ventricular muscle function is more difficult to evaluate. Tallifer R et al 1989. "Same day injections of Tc^{99}mm methoxyisobutylisonitrate for myocardial tomographic imaging; comparison between rest-stress and stress-rest injection sequences" European Journal of Nuclear Medicine, 15, 113-117.

A different approach employs an indium 111 labelled antimyosin which binds to xposed myosin following myocardial infarction. Although useful in diagnosing myocardial infarcts after cardiac surgery which suffers from the disadvantages that:

- it is not a blood pool imaging agent and will not evaluate vasculatur
- It furth r requires myosin to b available for binding

Khaw B et al 1987 "Acute myocardial infarct imaging with indium labelled monoclonal antimyosin fab" Journal of Nuclear Medicine 28, 1671-1678.

Khaw B et al 1987 "Myocardial damage delineated by indium 111 antimyosin fab and technetium^{99}mm pyrophosphate" Journal of Nuclear Medicine 28m 76-82.

A more useful approach is the labelling of platelets using Tc^{99}mm labelled P256, which is an anti-platelet antibody directed against fibrinogen receptors. Radiolabelled platelets image thrombus or those areas where platelets aggregate. Peters et al "Imaging thrombus with radiolabelled monoclonal antibody to platelets" Br. Med. Journal 293. 1525-1527.

The tendency of Tc^{99}mm P256 to filter through the kidney and the stickiness of platelets in certain circumstances make this agent less ideal for perfusion studies than labelled red cells.

The radiolabelling of erythrocytes is an especially desirable goal for reasons that:

a. Red cells remain in the vascular space
b. Oxygen supply directly follows red cells

Traditional techniques for the labelling of red cells involved the complex process of 'tinning; the red cell either *in vivo* or *in vitro.*

It was found that red cells exposed to the stannous ion subsequently took up pertechnetate. The discovery was exploited in two ways:

a. The *in vitro* method in which autogenous blood was tinned *in vitro* in citrate buffer and then labelled with Tc^{99}mm *in vitro* and re-injected - which carries the disadvantage of inconvenience, time, and undue manipulation of radionucleotides.

b. The *in vivo* method in which a large quantity of toxic stannous ion is administ r d to the blood stream followed thereafter by an injection of pertechn tat - carrying the disadvantage of a high load of toxic stannous ion.

The agent Tc^{99}mm HMPOA (hexamethlpropylenamineoxime) is taken up by many blood cells and is more convenient than the above techniques. However, this agent is not specific for red cells and has greater specifity for granulocytes, it is also excreated in the gut, further granulocytes tend to leave the vascular space diminishing contrast.

A difficult organ in which to assess perfusion is the lung. Perfusion studies are important to assist in the diagnosis of pulmonary embolism. Although the agent Tc^{99}mm HMPOA may find a place, lung perfusion studies need to be supplemented by ventilation studies using either $Xenon^{133}$ or Technigas which is Tc^{99}mm labelled smoke. (Burch et al 1986 "technigas a new ventilation agent for lung scanning" Nuclear Medicine Communications 7, 865-871.

The need remains for an agent which will provide a useful cold spot image of lung perfusion derangements as in pulmonary embolism, without the need for ventilation studies.

The present disclosure provides novel blood pool imaging agents which overcome the above problems used either on their own or with newer SPECT techniques. SPECT or single photon emission computerised tomography is likely to revolutionise the field of nuclear medicine and improve both image quality and the range of applications of radiopharmaceuticals. At present SPECT techniques are limited less by technology than by cost. The agents of the present disclosure will extend the use of SPECT techniques to improve brain, lung and liver imaging and the imaging of other organs.

It is known that blood vessels may be successfully imaged using Magnetic Resonance even without contrast agents. Several factors make this possible; time of flight effects and phase ff cts. Time of flight ffects depend on the motion of blood in a tissue and the direction of motion in relation to the plane of magnetization. Blood flowing into a

plane of section exhibits an intense signal a phenomenon called flow related enhancement or paradoxical enhancement.

Phase effects are produced by the fact that two gradient echo pulses are unable to cancel each other precisely as in stationary protons for reasons of the motion of the fluid. The phase shift is normally proportioned to blood velocity. A problem arises in that blood flow through a vessel is not constant being fastest centrally and slower peripherally - occasioning different phase shifts. Moreover in the heart turbulence may produce unpredictable phase shifts and deteriorate image quality.

Compensation techniques may be employed to overcome this problem termed 'flow compensation' or 'motion artefact suppression techniques'. Nevertheless such compensations can only be achieved with inconvenience. A further problem encountered with MR angiography is the difficulty displaying vascular anatomy in tissue blocks. The imaging of vessels in a tissue section may be achieved using the techniques already mentioned, however, the imaging of a vascular tree such as the cerebrovascular system or coronary vascular system in its entirety using only MRI without contrast is challenging at the very least.

Many researchers acknowledge that although MRI images may be produced without resorting to contrast agents and although this is a theoretical goal in the development of MRI, contrast agents are necessary in order to maximally exploit the technology. Accordingly much time and effort has been expended in trying to develop MRI contrast agents to facilitate the imaging of vascular structures and pathology.

This notion is especially true for the myocardium which manifests no difference in intensity or MRI imaging between normal myocardium and ischaemic myocardium during the few hours after infarction. Accordingly the use of some MRI contrast medium is essential for a cardiac imaging of ischaemic myocardium early in the process. Wendland M F et al Inv Radiol 1991: 26: 5236-238 disclosed how the agents GD-DTPA-BMA and DY-DTPA-BMA could be used to image acute infarction ischaemia within only 2 hours of the event by MRI.

DTPA-BMA is a linear chelating agent which carries a charge of -3 until complex d the Gd^{+3} which renders the agent electro neutral (charge 0). This electron utrality facilitates its administration as a bolus and helps retain the agent in the vascular compartment. See Tweedle M F Invest. Radiol. August 1992 %2-6 Vol 27 for a discussion of the chemistry of the agent.

A further study by M Saeed, Wendland M and Higgins C evaluated Gd - DTPA - BMA and Dy-DTPA - BMA in the assessment of the extent of the myocardial injury and found that without the use of a contrast medium infarcts re-perfused or occlusive could not be distinguished from normal myocardium. However the agents successfully produced signal differences between normal and infarct re-perfused and occlusive in rats. Inv. Radiology V 26 Nov 1991 Suppl 52391-241.

The improved intravascular retention of the agent Gd-DTPA-BMA (electroneutral) over the more easily diffusible agent Gd-DTPA has been matched by attempts which go further and attain still greater levels of vascular retention. D Meyer et al Investig. Radiol. Suppl. Nov 91; Vol 26, 550-52 'Paramagnetic Dextrans as magnetic Resonance Contrast Agents' Meyer et al disclose dextran molecules of MW 20,000 conjugated to DTPA using the method of P Sadler and Harding CT. WO 85/05554. The higher molecular weight of the molecules makes intra vascular retention more likely.

Dextran of larger molecular weight 70,000 may also be labelled using TRITA ligands (amino benzyl tetraazacyclo tridecane - tetra acetate). Labelled macropolysachharides compare favourably with labelled albumin and exhibit lower side effects and greater relaxation: Niemi P et al Invest Radiol 1991: 26; 548-549.

On similar lines a novel macromolecule polylysine - $(DTPA\text{-}Gd)_{40}$ was shown to be especially useful in assessing the perfusion of lung and produced 200% signal difference between perfused and non perfused tissue.

Polylysine $(DTPA\text{-}Gd)_{40}$ remained in the intravascular compartm nt for 60 mins at least without leaking. Brach R et al Invest Radiol 1991: 26; S42-S45.

The ultimate goal for a blood pool ag nt must be 100% vascular r tention. Doucet D et al Invest Radiol 1991: 26; S46-S47 cam clos st to achieving this by attempting to directly label red cells themselves. Labelled red cells would remain for many days in th circulation, and directly measure effective perfusion. Further in the absence of haemorrhage, also worth imaging, red cells do not extravasate into the tissues.

Doucet succeeded in labelling red cells by lysing the cells in hypotonic saline and thereafter resealing them with hypertonic saline after allowing the red cells to absorb Gd-DOTA into their cytoplasm. Doucet et al's disclosure suffers from the major drawback that a separate laboratory procedure must be carried out on the red cells before imaging.

The present disclosure provides the advantage of a macromolecule and the advantages of red cell labelling without any laboratory procedures on red cells being required.

The novel labelled macromolecular agents of the present disclosure bind directly onto red cells in one step and represent a significant advance in techniques discussed herein before. The macromolecular agents of the present disclosure provide a novel use for modified peptides of the malaria parasite organism.

Of all parasites malaria must be the most damaging and successful. Many millions are infected with 1-2 million deaths per annum. The malaria parasite has evolved from earliest times and attacks not only humans but most varieties of animal. This serious parasite infests red blood cells. Various malaria species infect humans, *plasmodium falciparium*, and *plasmodium vivax* being the most important. The life cycle is complex with a short life cycle in the salivary gland of mosquitoes and following inoculation of a human the parasite objective is ultimately the red cell. Merozoites bind to the red cell membrane, enter the cytoplasm and multiply.

The course of malaria is a variable one and may be characterised by a short acute illness which can bring death in a matter of hours; or a longer more chronic illness associated with debility and anaemia.

Other forms of malaria such as the *plasmodium Knowlesi* are well researched animal parasites which infects the *rhesus* monkey. The preferred location for the malaria parasite is within the red cells of the infected host and for much of its life span it lives intracellularly protected from the host immune system.

Merozoites are thought to spend but a brief period free in the circulation. Accordingly, considerable research efforts have been expended to discover the means whereby the merozoite forms of the parasite gains attachment and gains entry into the human red cell.

Margaret E Perkins Journal Experimental Medicine 160 September 1984, 788-798 is Responsible for formulation of a relationship between the *plasmodium falciparum* binding molecule and glycophorin A and B two sialo glycoproteins found on the surface of red cells.

More laterally, Holt and Perkins et al American Journal Tropical Medicine Hygiene 1989, p245-251 disclose species and stain variations of *plasmodium falciparum* wherein some strains of the organism exhibit preference for sialo glycoproteins glycophorins A, B and C and also demonstrated was the varying requirements for the N-acetyl-neuramic acid residues (NeuNac).

The glycophorin A molecule is a highly glycolated peptide. Pasvol has suggested that glycophorin binding peptide binds to the region of glycophorin close to the lipid bi-layer.

Pasvol G et al "Inhibition of Malaria Parasite Invasion of Monoclonical antibodies against glycophorin A correlates with a reduction in red cell membrane deformality." Blood, 74, No. 5, October 1985, 1836-1843.

Debate continues within the literature as to the requirement for sialic acid on the glycophorin molecule to affect invasion by merozoites.

Some strains of malaria are totally dependent on normally sialated glycophorin A to gain entry into the red cell, whereas other strains seem to be independent of sialic acid. See Mitchell et al 67, No 5, May 1986, 1519-1521. Perkins and Roco in Journal of

Immunology, 88, Vol 141, 3190-3196, No 9, again stress th importance of sialic acid where normally sialated glycophorin is necessary to achiev successful binding of merozoite peptides in particular pf200.

For several years a peptide called the glycophorin binding protein was believed to be the primary peptide responsible for binding merozoites to erythrocytes. A gene coding for GBP was isolated by Perkins M Ravetch J and Kochan J and disclosed in Science Vol 227, p1593-1596, 29 March 1985 and incorporated herein fully by reference.

GBP 130 is characterised by a tandem repeated sequence coding for a 50 amino acid repeating sequence believed to be the site of erythrocyte binding, "A tandem repeated sequence determines the binding domain for an erythrocyte receptor binding protein of *plasmodium falciparum*". Cell, Vol 44, 689-696, March 14, 1986, Kochan J, Perkins M and Ravetch J. See Figure 2, p691, which also discloses the full sequence and genetic code of the GBP 130 molecule.

Other workers have challenged the supremacy of the GBP 130 as the primary binding molecule of the malaria merozoite. Orlandi P, Kim Lee Sim et al Molecular and Biochemical Parasitology, 40 (1990) 285-294 "Characterisation of the 175 kilodalton erythrocyte binding antigen of *plasmodium falciparum*" suggested a different peptide, the EBA 175 molecule, as being responsible for merozoite binding or at least playing some role thereto.

The EBA 175 molecule like the GBP 130 molecule has an affinity for the red blood cell surface and binds therein.

It is known that the EBA 175 molecule has a prediction for olygosaccharides which are found on the surface of the red cell molecule. However, a problem arises in that the EBA 175 molecule does not bind effectively with the malaria merozoite parasite. Therefore, it is thought that the EBA 175 serves a function as a bridge. This disclosure proposes an alt rnative mechanism in that the EBA 175 molecule is responsible for bringing the merozoite close to the erythrocyte membrane surface, thereafter GBP 130 drags the merozoite closer still by binding with the base of the glycophorin A peptide; thus bringing

the lipid bi-layer of the malaria parasite into approximation with the lipid bilayer of the red cell membrane and thereby allowing the incorporation of the parasite into th rythrocyt itself. This disclosure suggests the merozoite is winched into the RBC cytoplasm.

The genetic sequence and the peptide sequence of EBA 175 was disclosed in J. Cell Biology 111, 1990, Kim Lee Sim, Orlandi P et al "Primary structure of the 175 Kd *plasmodium falciparum* erythrocyte binding antigen and identification of a peptide which elicits antibodies that inhibit malaria merozoite invasion" See Cell Biology Vol 111 (1990) p1877-1884, Figure 2 of p1880 for the sequence of amino acids and DNA sequence.

To further complicate the picture of Dogmar Nolte et al described two close relatives of the glycophorin binding peptide 130 molecule which they call GBPH or glycophorin binding peptide homologues. This molecule like the GBP molecule, displays several tandem repeat sequences and a high affinity for the erythrocyte surface membrane surface peptides. It has been proposed by Nolte and co-workers that it is the GBPH molecule and not the GBP molecule that is responsible for erythrocyte binding of the parasite in that the GBP molecule is released as an immunogenic decoy to distract the immune system from the real binding peptide the GBPH.

The nucleotide sequence of one form of the peptide GPBH is disclosed by Dagmar Nolte et al in the Journal of Molecular and Biochemical Parasitology, 49, (1991), p253-264. See Figure 2 of p 257 incorporated herein fully by reference. The peptide sequence is also disclosed.

See also Figure 3 of p 258 the same journal and paper which lists a comparison between GBP 130 and GBPH. Binding and entry of merozoites into RBC's involves several peptides or several alternatives as fail safes for the organism.

The picture is further complicated by other research notably by Peterson Gregory, who proposes PMMSA (Pre major merozoite surface antigen) as being responsible for erythrocyte binding either in this state or following fragm ntation into smaller fragments. The genetic sequence and the peptide sequence of th PMMSA mol cul is given in the

Journal of Mol cular and Biochemical Parasitology, 27 (1988), 291-302. Se Figure 3 of p294 and 295. Peterson G et al.

Erythrocyte binding using different peptides and surface molecules is exhibited by other species of the malaria parasite in particular the *plasmodium vivax* organism. This organism can infect only persons expressing the Duffy marker. The Duffy antigen is a red cell surface marker and is one of many blood group markers and is carried by a percentage of the population.

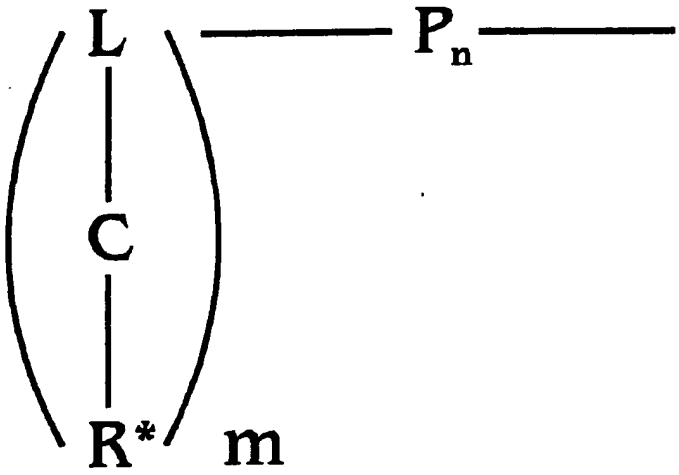
Persons not expressing Duffy antigens are therefore immune from infection by *plasmodium vivax*. The *plasmodium vivax* expresses a Duffy binding receptor molecule *P. vivax* Duffy receptor was cloned and sequenced by Xiangdang Fang and disclosed in Molecular and Biochemical Parasitology, 44 (1991) p125-132. See especially Figure 1 of p127 for the genetic sequence and amino acid sequence.

Similar to *plasmodium vivax* is *plasmodium Knowlesi* which also uses the Duffy antigen. This organism parasitises *rhesus* monkeys. Also in the same Journal, same figure, same page, is listed the genetic sequence of *plasmodium Knowlesi* Duffy receptor molecule which may find a use in the agents of the present disclosure.

When developing therapeutic agents directed against the malaria parasite itself, then it is clearly important to identify the precise molecule responsible for merozoite binding in the clinical context. However, where malaria peptides are to be employed as erythrocyte binding agents more generally, then it is not important to identify the precise peptide the malaria organism uses to effect invasion. Any malaria peptide capable of binding to an erythrocyte surface membrane may have a therapeutic use for other purposes such as the agents of the present disclosure and also segments of such a peptide.

The novel agents of the present disclosure are blood pool imaging agents formed by peptides all or part derived from the merozoite of malaria parasites having affinity for human red cells and the said macromolecules to be labelled either directly via complexing with a chelating agent the said chelating agent to be labelled by a lab lling group such as a radioactive or paramagnetic group or element; or the said

macromolecules to be labelled indirectly by fusion to a polypeptide linking sequ nce such as d scribed herein. The macromolecules may also be lab lled by labelling agents such as described herein. The macromolecules may also be labelled by radio-opaque groups usually iodinated for use in conventional CT imaging. The general formula for the imaging agents being:



Where P is all or part or a polymer of segments of all or part of a merozoite derived polypeptide such as GBP130, GBPH, PMMSA, EBA175 or *P. vivax* Duffy receptor;

Where n is a natural number;

Where m is a natural number;

Where L is a polypeptide linking sequence joined to P either C or N terminally and where L may be of the type

(Xaa)m

(Zaa)m

(XaaZaa)m where Xaa and Zaa are amino acids and especially polylysine, or even single amino acids such as serine;

Where C is a chelating agent especially but by no means only

DTPA

DTPA BMA

DOTA

HP - DO3A

EDTA

EGRA

TTHA

HBED and the like;

And where R* is a labelling moiety preferably but by no means limited to

$Tc^{99}m$

I^{132}

In^{III}

I^{131}

Ru^{97}

Cu^{67}

Ga^{67}

Ga^{68}

Au^{145}

for radio labelling or the paramagnetic elements

Gd 157; Mn 55; Dy 162; Cr 52; Fe 56;

or the fluorescent element Eu 152.

BRIEF SUMMARY

The field of the present disclosure relates to bioradio pharmaceuticals/imaging molecules. Bioradio pharmaceuticals and other imaging molecules are used in the field of diagnostics to form an image of pathology or to demonstrate the physiological function of an organ being investigated such as its blood flow. In general bioradio pharmaceuticals or imaging peptides are formed by the fusion of a radio isotope or paramagnetic group to a peptide carrier by means of a ligating agent. The disclosure also provides contrast agents where the labelling moiety is a halogenated compound.

Vascular disease counts for the largest simple category of pathology ranking third after infection or neoplasms.

Vascular disease may take three forms:

i) haemorrhage or leaking
ii) blockage of a vessel or restriction of a vessel
iii) formation of new vessels such as haemangiomas.

In all cases it may be necessary or desirable to form an image of the abnormal blood flow to the particular part.

The most frequent examples of life threatening vascular pathology are deep vein thrombosis of the leg, pulmonary embolism and coronary artery thrombosis. Pulmonary embolism is a feared complication of surgery. It is estimated that some 100,000 US citizens per year die from consequences of pulmonary embolism alone. Moreover, the diagnosis of pulmonary embolism may be particularly difficult.

Several approaches of diagnosing pulmonary embolism are available in the present art such as ECG, which suffers the disadvantage of vagueness and inconclusiveness. Other approaches involve pulmonary arteriography which carri s the side eff cts and complications associated with all arteriography. Other approach s involve th us of

radio isotopes labelling HAA (Human Albumin Aggregates) which also carry th sid effects of adverse reactions to the small albumin aggregates.

Still further approaches involve the uses of technetium^{99} labelled human albumin aggregates used in conjunction with ventilation studies involving radio active gases. Such combination studies are referred to as V/Q scans or ventilation perfusion studies. Traditionally ventilation being assessed using radioactive argon.

More recently, technetium^{99} has been used to radio label ' smoke' atomised carbon "techni gas".

The present disclosure offers improvement on existing techniques and provides a means of diagnosing pulmonary emboli in one step. Other pathological conditions will benefit from the diagnostic imaging techniques provided by the disclosure and include cardiac failure in which the left and right ventricular ejection fraction can be determined; myocardial infarction in which it may be advantageous to determine the patency of coronary arteries or the blood flow rates to cardiac muscle. Still other organs benefit from blood flow determination eg. brain and lower limbs both pre and post arterial surgery. Not infrequently, halogenated blood flow and assessment of vessels are determined by means of halogenated contrast media ie. arteriograms which suffer some disadvantages. The use of contrast media where radio opaque substances flow freely in the blood stream is known to be associated with a significant morbidity and mortality undesirable in a diagnostic procedure.

In an attempt to reduce the morbidity involved in diagnosis, hopes have been pinned on the use of nuclear magnetic resonance. Nuclear magnetic resonance is a technique in which the body is subjected to a powerful magnetic field; on release of the magnetic field, aligned molecules release radio frequencies, these radio frequencies are then used to form an image. A flow of blood within a vessel generates the phenomenon of 'time of flight' effects. These 'time of flight' effects enable contrast to be developed.

Contrast is achieved by the stronger signals produced by rapidly moving blood especially when the blood is flowing in certain directions relative to the radio frequency receiver and

magnet. Both 'time of flight' and 'phase contrast' effects rely on the magnetic prop rties of moving fluids and may produce excellent images in good hands without the ne d for contrast agents. However, vascular imaging by MRI with these techniques alone and without contrast agents is time consuming and impractical in many cases. Alternatively, molecules containing paramagnetic groups or ions can be used to increase the contrast between vessels and surrounding tissues in NMR angiography. Examples of such compounds are compounds of gadolinium which are used especially in cerebral vascular NMR angiography.

An area of active interest at the present time is the development of bioradio pharmaceuticals. A bioradio pharmaceutical may be considered to be a radio nucleotide joined to a peptide sequence or other bioactive molecule designed to bind to specific receptors or cells. Examples of such compounds would be P256 which consists of a technetium^{99} antiplatelet labelled antibody. The purpose of this compound being to bind with fibrinogen in deep vein thrombosis. P256 may also be used to demonstrate platelet restenosis following coronary artery bypass surgery.

Other agents useful for imaging deep vein thrombosis are E1 a fibrin polymer fragment which is also labelled with radio nucleotides and technetium^{99} HMPAO labelled platelets.

Still other workers have reported the use of radio labelled melanocyte stimulating hormone, used for the purpose of determining the extent of melanoma. Technetium^{99}m HMPAO (Hexa Methyl Propylene Amine Oxime) may be used to label white blood cells and thereby demonstrate the site of infection sometimes useful to determine the source of a PUO (Pyrexia of Unknown Origin).

Despite these advances the radio labelling of red blood cells themselves remains as yet unsatisfactory.

Blood pool determination and imaging is especially useful for determining dynamic heart function. Conventionally, Tc^{99}m HSA or Human Serum Albumin is used. This is carri d .out by compl xing p rtechnetate after it's reduction with albumin, the r duction being achi ved using either a platinum or zircronium lectrode or by stannous chlorid with acid.

$Tc^{99}m$ suffers the disadvantage of rapid blood clearance. Images are possible only for 1 hour after injection. Further disadvantages are the development of impurities contributing to background activity and degrading the desired image.

To overcome these defects inherent in $Tc^{99}m$ HSA, workers have tried to radiolabel RBC red cells directly. Two techniques are used at the present time.

The Brook Haven technique or *in vitro* technique involves the 'tinning' of red cells in a vacutainer containing stannous ion 2.0μg, citrate; dextrose and NaCl. Thereafter 1.25ml of tinned red cells are added to a vial containing pertechnitate. After 10 minutes, a 98% labelling yield is achieved and red cells administered.

The drawback with this system is it's complexity and inconvenience. The *in vivo* method was discovered by accident.

In this technique radio labelling occurs *in vivo* but only in the presence of significant amounts of stannous ion 7.4mg of stannous pyrophosphate per 100ml of blood are usually required. Labelling yields of 70-80% are usual.

The major advantages of this technique are:

1) Convenience
2) Use in first pass studies.

The disadvantages are:

1) Rapid diffusion of agent and
2) Concentration by gastric mucosa
3) Large amount of stannous ion.

The present disclosure contains all the advantages of the *in vivo* labelling method with few disadvantages. The compositions of the present disclosure further provide very high

labelling yields *in vivo*; the possibility of using non nuclear means of imaging; absence of stannous ion containing steps; and most importantly very low rates of diffusion; with ease of use; and good image quality.

The present disclosure teaches a novel method of radio labelling the red blood cell molecule; thereby permitting the said radio labelled or paramagnetically labelled red blood cells to function within the context of nuclear imaging or paramagnetic resonance imaging, or conventional scintigraphy with a gamma camera or other device, or conventional CT scanning.

The present disclosure envisages the ligating of radio isotopes or paramagnetic groups to malaria derived peptide sequences, the said peptide sequences having the ability to bind to receptors on the surface of human blood cells. Examples of such chemical ligating agents include:

- DTPA: diethylenetriamine pentacetic acid
- EDTA: ethylenediamine tetracetic acid
- EGRA: ethylene glyco-o, o-bis (2-aminoethyl) - N, N, N^1, N^1 - tetracetic acid
- HBED: N, N^1 - bis (hydroxybenzyl) ethylenediamine - N, N^1, - diacetic acid.

The ligating agents may be like one or more in any combination of the following peptide sequences:

a) A peptide sequence comprising amino acid residues 201-774 glycophorin binding peptide 130 molecule or part thereof; or multiples thereof or polymers of tandem repeating segments there from.

b) A peptide sequence comprising amino acid residues 1-774 or of the glycophorin peptide binding 130 molecule or fragments thereof.

c) A single tandem repeat sequence of the glycophorin peptide 130 binding molecule or repeated fragments ther of.

d) A peptide sequence derived from the glycophorin peptide binding homologu molecule GBPH or r lated molecul s, amino acids sequ nce 70-427 or fragm nt thereof.

e) Sequence derived from the glycophorin peptide binding homologue molecule or fragment therefor comprising amino acids 109-427 or fragments thereof.

f) A peptide sequence derived from the *plasmodium vivax* Duffy Receptor molecule comprising amino acids 23-1051 or fragment thereof.

g) A peptide sequence derived from the erythrocyte binding antigen 175 (EBA-175 molecule) comprising amino acid residues 20-1435 or fragment thereof;

h) A peptide sequence derived from the expression of the nucleic acid sequence derived from the expression of the nucleic acid sequence or fragment thereof derived from the nucleic acid sequence encoding the pre major merozite surface antigen molecule PMMSA molecule residues 177-5169 or fragment thereof;

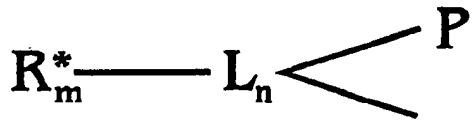
i) A peptide sequence derived from the *plasmodium Knowlesi* Duffy Binding Receptor molecule or fragment thereof.

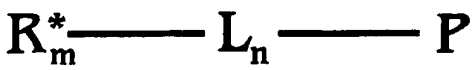
The said peptide sequences be ligated by means of the chemical ligands disclosed hereinbefore to radio nucleotides as follows:

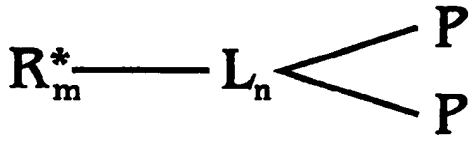
^{125}I, ^{131}I, ^{90}Y, ^{67}Cu, ^{217}Bi, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd for radiotherapy
^{3}N, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Ca, ^{75}Se
^{99}mTc, ^{132}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{195}Au, for *in vivo* imaging

is also envisaged that paramagnetic groups or elements may be ligated to the said peptides. Useful paramagnetic elements but by no means the only useful paramagnetic elements are:
^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, ^{56}Fe or the florescent lement ^{152}Eu

General formulae for the agents of the present disclosure are:







Where R^* is a radionucleoide or paramagnetic or labelling group;
L is a chemical linker;
P is a peptide sequence
and where m, n and x are natural numbers
and where L may be a chelating agent or a chelating agent bound to a linking peptide such as polylysine.

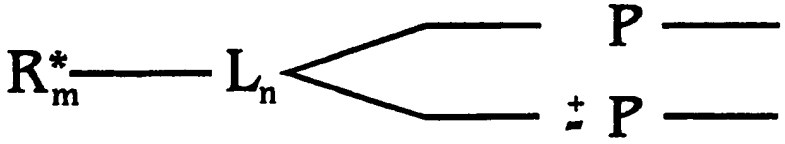
It is also envisaged that the molecules of the present disclosure may be used in conjunction with positron emitting atoms.

Carbon - 11; Oxygen - 15; Nitrogen - 13; and the like,

incorporated into other groups which will combine with the chemical ligands used in conjuctions with peptide sequences, referenced herein before. It will be appreciated by the skilled artisan that any labelling groups other than those mentioned may be used with th p ptide sequences herein disclosed without departing from th scope or spirit of th invention.

EXAMPLES:

The disclosur may be illustrated by the following non limiting examples derived from the general formula:

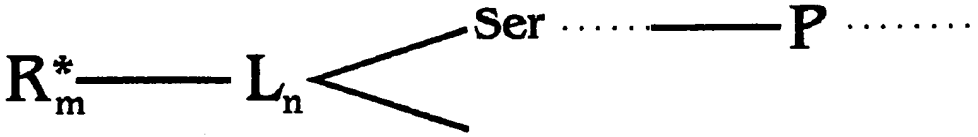


Where R is a radionucleotide or paramagnetic atom or group chosen from those described;

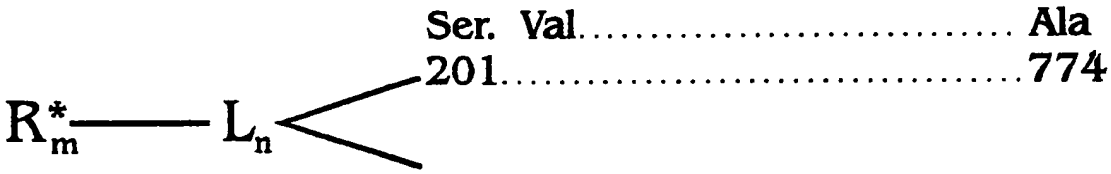
L is a linker such as DTPA;

P is a peptide chain; and m and n are natural numbers one or more.

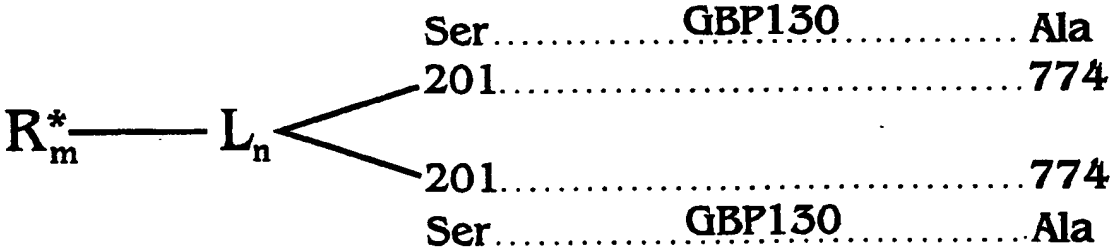
It may be preferable to insert a serine residue between the peptide chain and the chemical linker thus producing the novel composition:



Suitable peptide sequences may be derived from the glycophorin binding peptide GBP 130 as disclosed by Kochan J et al in Cell Vol. 44; 689-696. See Figure 2, Page 691; producing by way of examples.



or

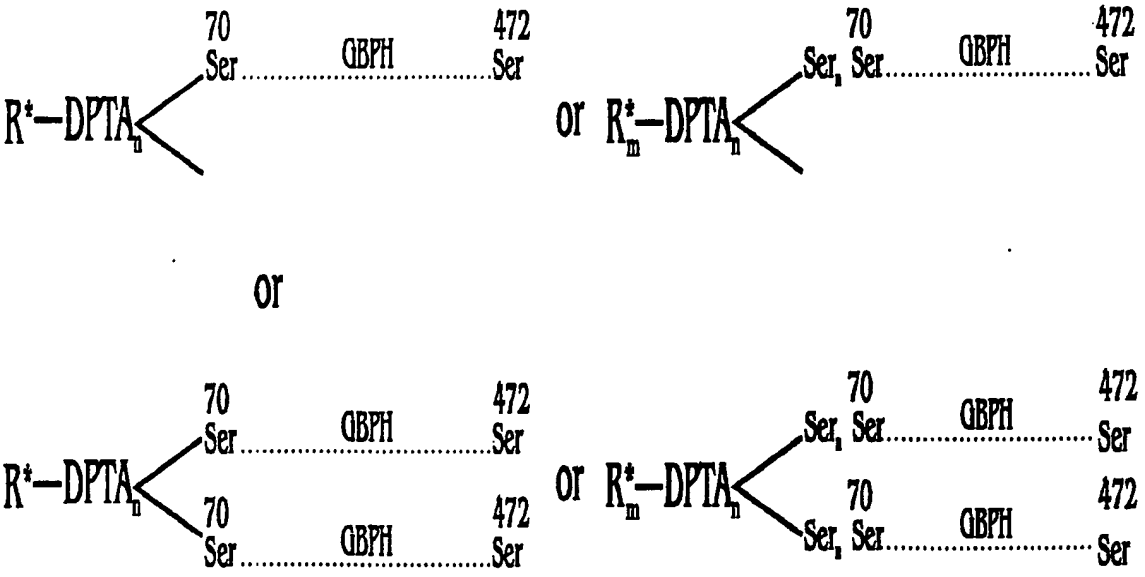


Where m, and n are natural numb rs.

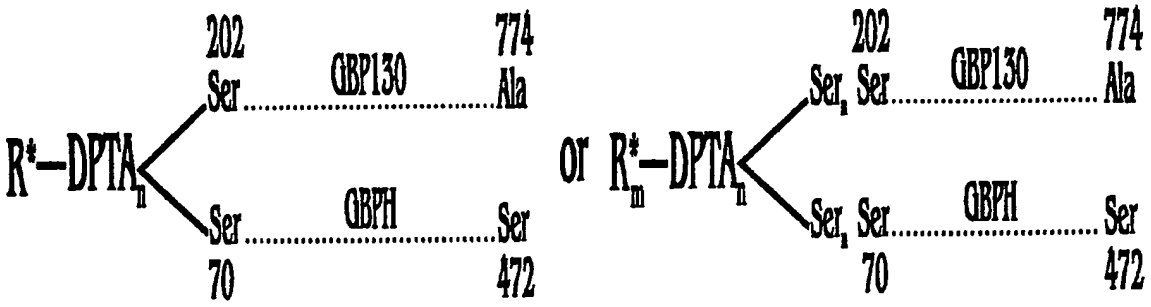
Suitable sequences of the GBP 130 molecule but by no m ans th only sequences are: amino acid residues 201-774; 202-774, residue 202 is a serine; or fragment thereof: or a shorter fragment such as a single tandem repeat for example 370-426; or polymers of a tandem repeat segment.

It will be appreciated that any GBP 130 sequence or substituted or insertional or deletional variation thereof may be used to fabricate the peptides of this disclosure, provided it can bind to erythrocytes.

Other useful peptide sequences may be derived from the GBPH molecule otherwise known as the glycophorin peptide binding homologue molecule as disclosed by Nolte in Molecular and Bioch. Parisitology, 49 (1991), Page 253-264. See Figure 2 of page 257.



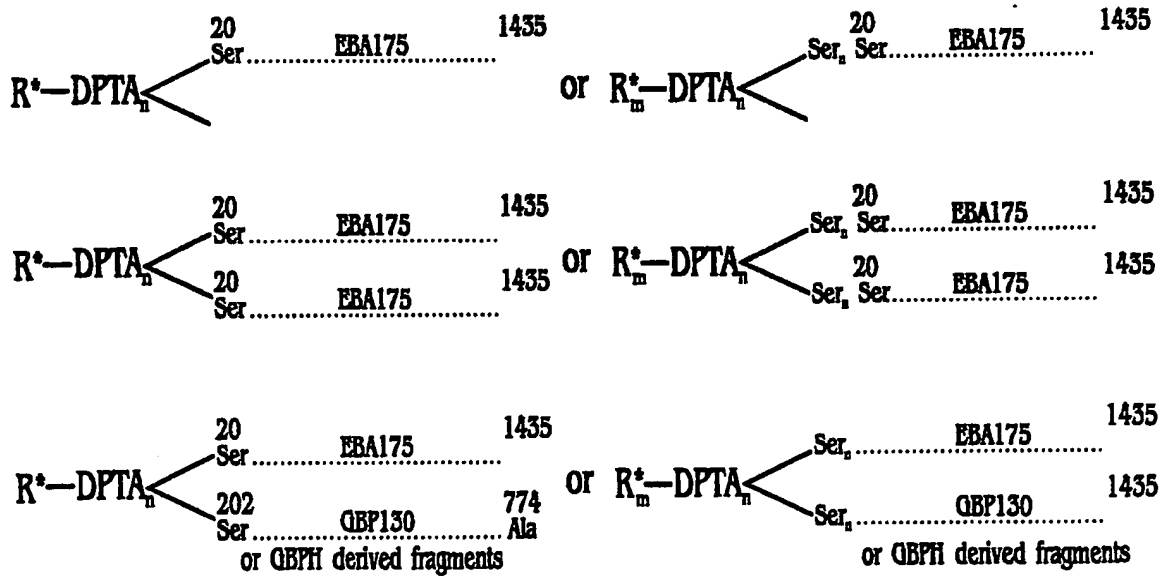
or in a combination with GBP 130 sequence



Suitable sequences are the peptide sequence of GBPH, Ser 70 to Ser 427 or fragment thereof; or the sequence 109 to 427 of the GBPH molecule or fragment thereof or the smaller fragment 230 to 268 comprising a tandem repeat, or any tandem repeat or polymers of a tandem repeat.

Other useful peptides for the present disclosure are sequences derived from EBA-175 or erythrocyte binding antigen 175 as disclosed by B Kim Lee Sim et al in J. of Cell Biology Vol. III, 1990, p1877-1884. See Figure 2, page 1880.

Producing by way of example



Suitable sequences derived from EBA 175 are: the sequence of amino acids 20-1435, or fragm nt thereof or smaller fragment comprising amino acid residu s 1062 - 1103.

It will be appreciated that any malaria derived peptide or peptide fragment or substitutional or deletional or insertional variant thereof possessing the ability of binding with a RBC molecule may be used for the novel compositions of this disclosure.

It will be still further appreciated by these skilled in the art, that anti-ideotypes of GBP 130; GBPH; EBA 175; *P vivax* Duffy receptor and the like could be used to manufacture the imaging agents of the present disclosure. These substitutions being obvious to the skilled artisan are not illustrated herein in the interests of brevity but fall within the scope and spirit of the present disclosure.

The peptides may be manufactured by Merrifield synthesis or by DNA cloning and assembled by means known to the art.

Purification of the peptides and compositions of this disclosure may be carried out by conventional techniques such as chromatography.

DETAILED DISCLOSURE

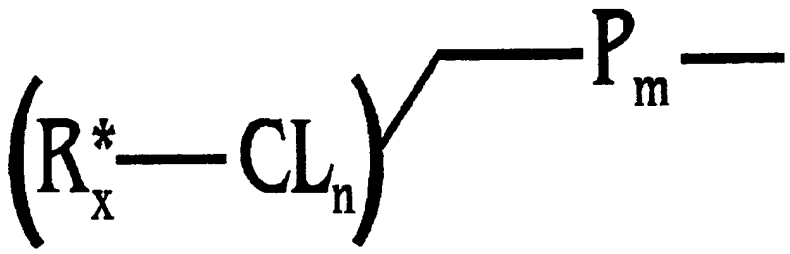
The disclosure is best illustrated by reference to th exemplary embodim nts described herein after which are non limiting.

EXEMPLARY EMBODIMENT GROUP 1

In this embodiment all or part of the peptide sequence comprising amino acid residues 201-774 of the GBP 130 (glycophorin binding peptide molecule 130) all or part or substitutional or deletional variations thereof or fragments thereof especially tandem repeats or modified fragments thereof are modified to form blood pool imaging agents as shown herein below.

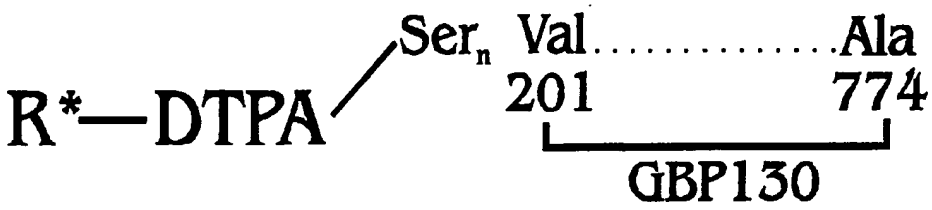
The amino acid sequence of the glycophorin binding peptide 130 molecule was disclosed by Jarema Kochan et al in Cell Vol. 44: 689-696, March 14; 1986. See Figure 2, p691 and is incorporated fully herein by reference.

The general formula for the agents is as follows



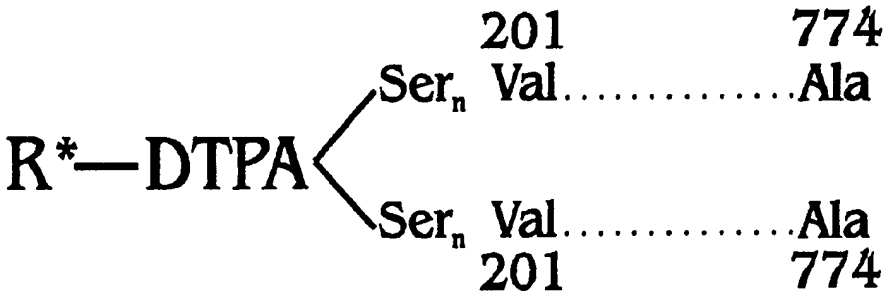
Where R^* is a radioneucleotide or paramagnetic group or labelling moiety
CL is a chelating agent - Linker complex
P r presents GBP 130 or fragment th reof
and where m and n are integers 1 or more, also x

I.(a).1



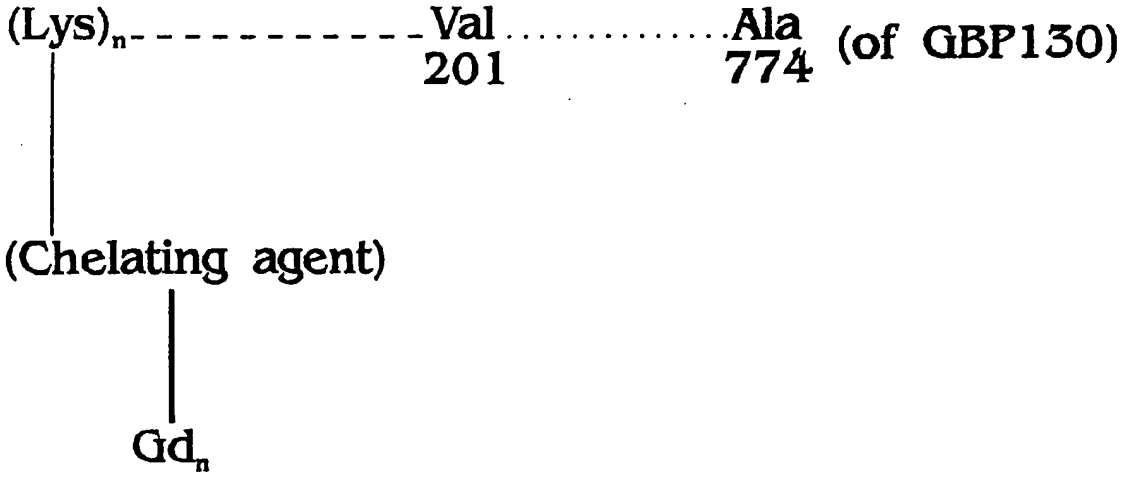
When n is 1 or more

I.(a).2 (see Figure 11)

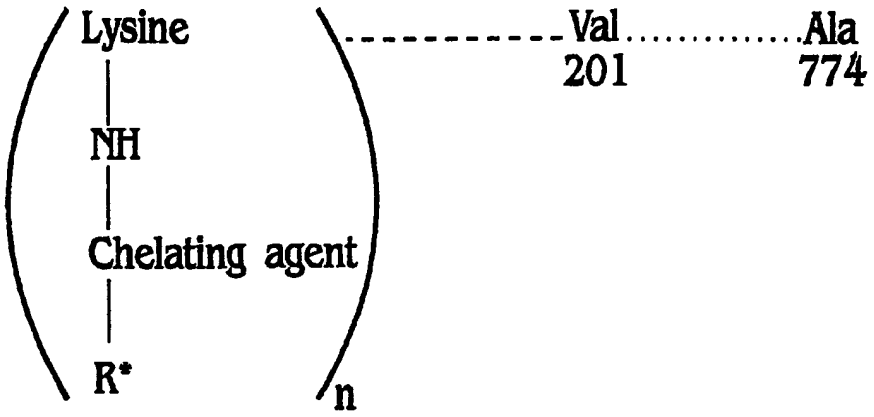


Where n is one or more and m is one or more

I.(b).



I.(b).1

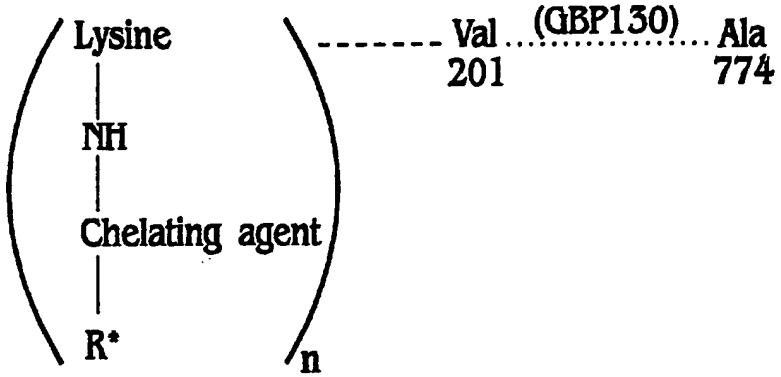


Where n is a real number; preferably up to 40;

Where the chelating agent may be; DTPA or a similar chelating agent

and where the labelling group may preferably by Gd or Dy or Yt; or any previously mentioned labelling moiety.

I.(b).2



Where n is a natural number preferably up to 50

Where Xaa is an amino acid not necessarily the same amino acid

Where m is a natural number preferably 2 or more

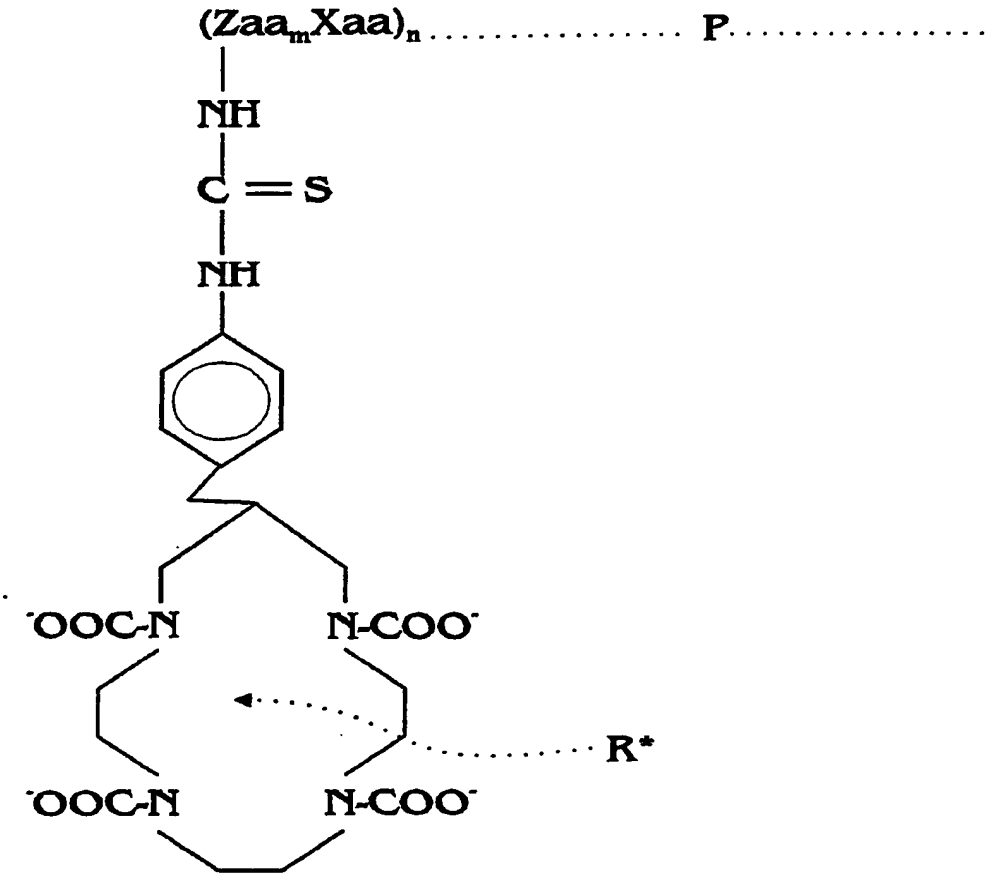
Where the chelating agent is any mentioned preferably DTPA; and also EDTA; EGRA; TTHA; HBED.

Where R is any mentioned labelling group preferably the group Gd; or Dy; or Yt or those others previously referred to, especially ^{55}Mn, ^{52}Cr, ^{56}Fe, in the case of paramagnetic labelling and ^{99}mTC, ^{132}I, ^{111}In, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga, ^{195}Au, where radio imaging is desired using a gamma camera or similar device.

I.(c).i.

Th TRITA agent may be us d in place of DTPA and a polypeptid linker oth r than lysine may be used to bind the TRITA - R^* to the peptide.

The general formula for such an agent being



Where Xaa or Zaa are the same or different amino acids at least one of which is capable of condensing with the amino group of TRITA, and may be lysine.

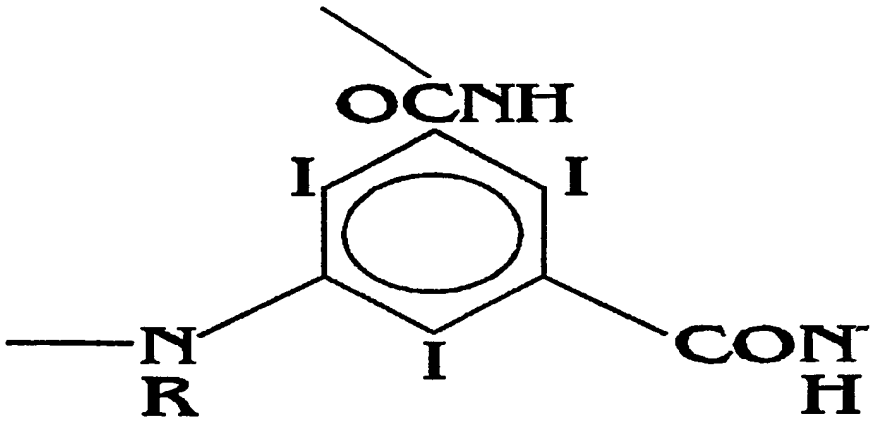
The use of TRITA agent to couple Gd to dextran was disclosed by Pekka Niemi et al Invest Radiol 1991 26; 548-549 and is incorporated fully herein by reference.

In this embodiment P is taken to mean segments of the GP 130 molecule all or part thereof.

The subscripts m and n ar natural numbers.

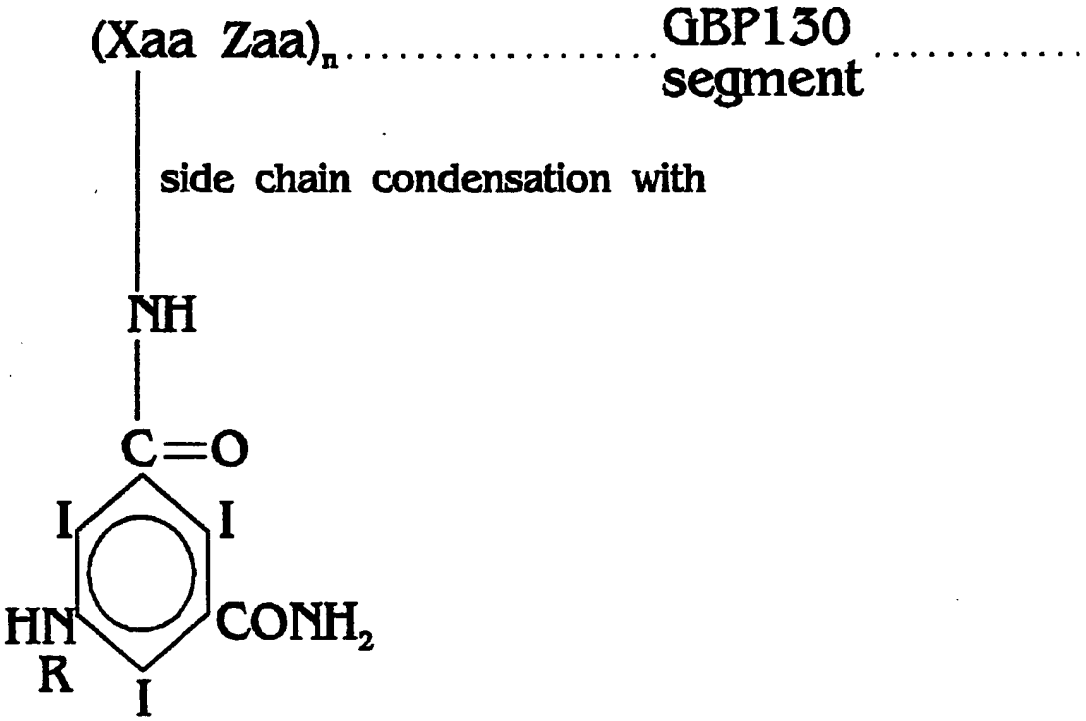
I.(d).

Iodinated carboxamides of general formula



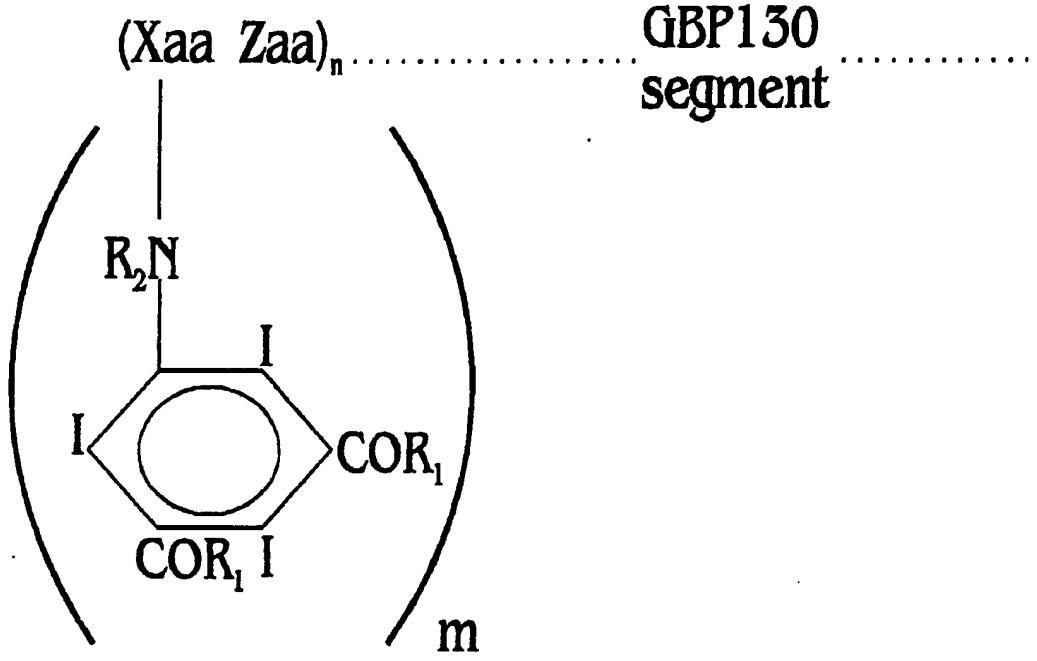
May be condensed with amino acids Zaa or Xaa in polypeptide $(Xaa\ Zaa)_n$; the said polypeptide to be fused to segments or all of the GBP 130 molecule thereby generating the iodinated agent as illustrated in I.(d).1 useful for CT scanning.

I.(d).1



I(d).2

It will be appreciated that side chain condensation reactions may also take plac with the Nitrogen in 5 position



Where R_2 is preferably H or a double bond

m < n and 'm' and 'n' are integers one or more

R_1 is probably NH_2

ie.

Newer chelating agents capable of binding with side chains of polypeptide linkers such as polylysine or other peptide polymers are:

HP-DO3A (Bristol Meyers Squibb)

DOTA (Guerbet)

DTPA-BMA (Sterling Winthrop)

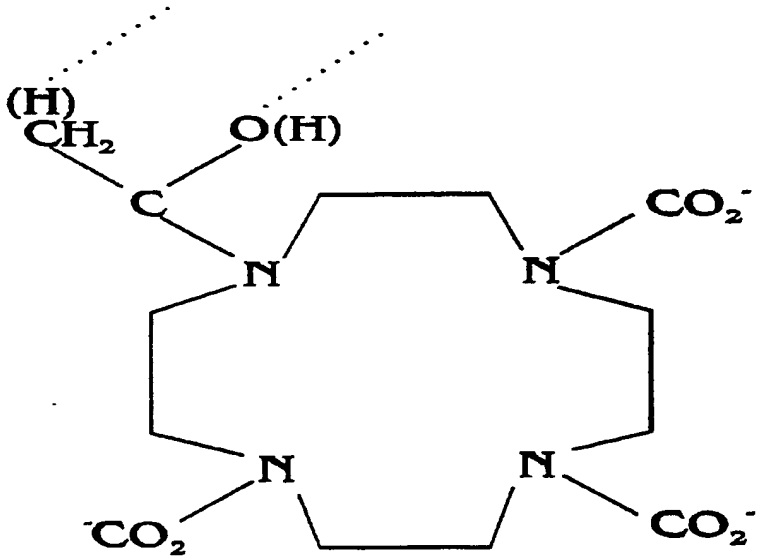
Non limiting examples of the use of such chelating agents are given herein below.

I.(e).1

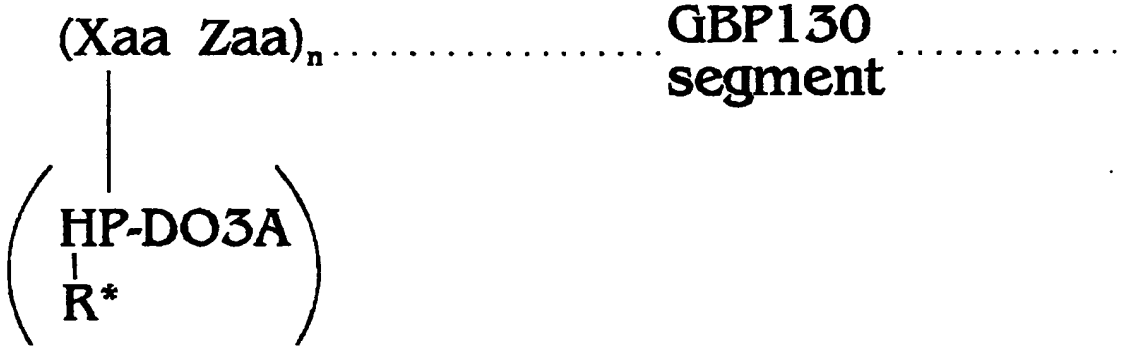
The chelating agent HP-DO3A (Bristol Meyers Squibb) b ars a charg of -3 producing a net charge of zero when complexed with Gd^{3+}.

Electroneutrality is preferred in polymers designed to carry multiple labelling moieties.

Accordingly binding to an amino acid side chain should preferably be via the methyl group or the hydroxyl oxygen.



Thus producing



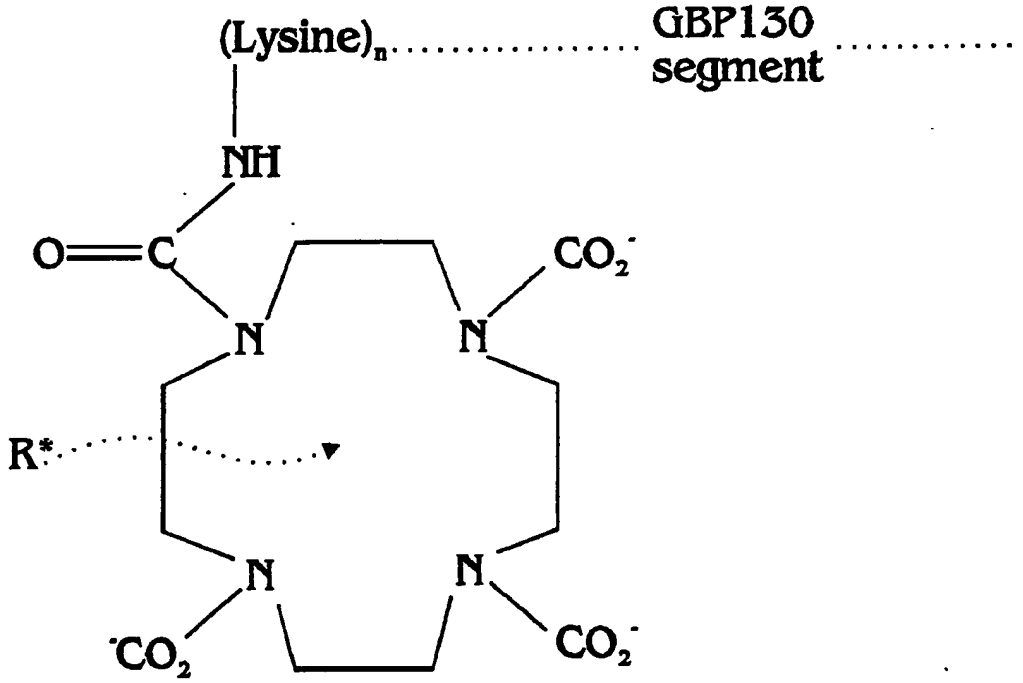
Where R* is any labelling moiety by especially Gd or Dy where paramagnetic contrast agents are desired or a radioactive moiety such as $Tc^{99}m$ where a bioradiopharmaceutical is desired.

I.(e).2

The DOTA chelating agent (4-) may be reacted usefully with the side chain of polylysines to give DOTA (3-) which complexing after Gd^{3+} becomes electroneutral which has the advantages already described.

DOTA was developed by Guerbet and their disclosures are incorporated fully herein by reference.

The agents so produced are of the type

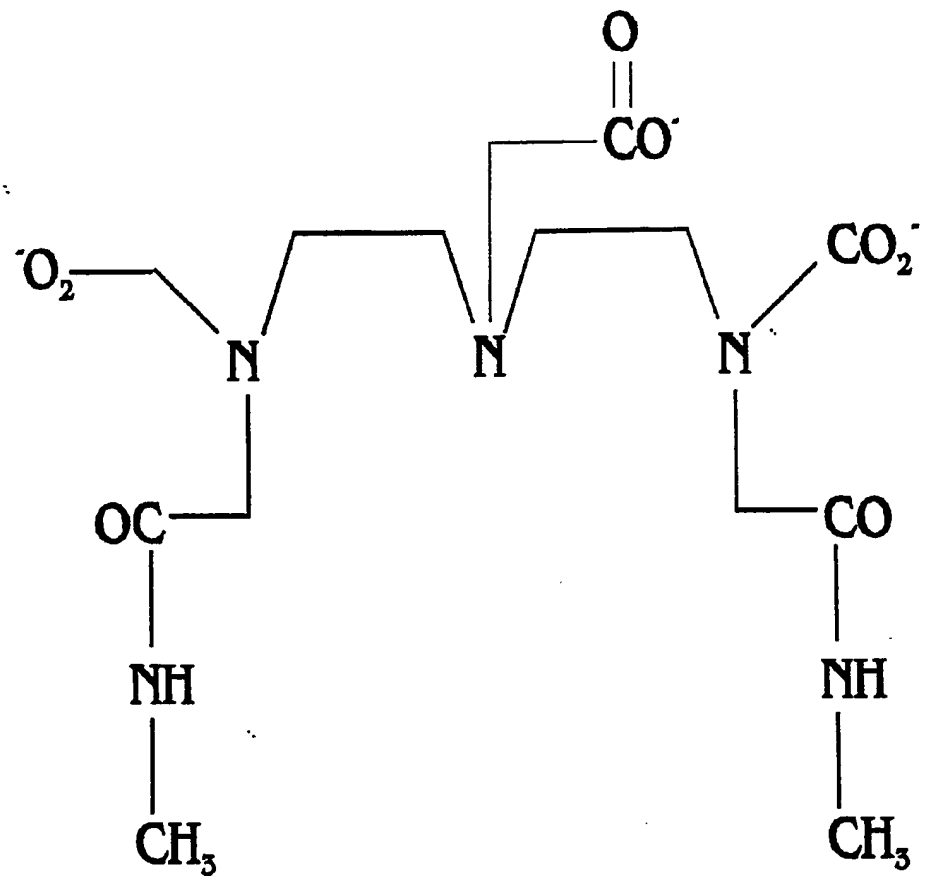


DOTA

Where R^* is preferably but not necessarily Gd^{3+} other amino acid linkers may be used in place of $(lysine)_n$

I.(e).3

The chelating agent DTPA -BMA developed by Sterling Winthrop of formula



May be reacted with $(Xaa)_n$ poly amino acid linkers, side chain thereof, via a choice of carboxylic residues of which there are three or via the aminated carboxyl carbons producing agents similar to these herein before described.

I.(f).

It will be appreciated that the orientation of the GBP 130 molecule may be reversed in any of the foregoing embodiments and that the R^* - < (labelled linker) complex may be joined to the merozoite peptide fragment at either the - NH_2 or COOH terminal end, or in the middle, say between tandem repeats.

EXAMPLE OF AGENTS GROUP 2

The agents of group 2 use the Glycophorin binding peptide homologue molecule for red cell binding.

The glycophorin binding peptide homologue molecule was cloned and disclosed by Dagmar Nolte et al Molecular and Biochem Parasitology, 49 (1991) page 253-264. See especially Figure 2, p257 and is incorporated fully herein by reference.

Example 2(a); 2(b); 2(c); 2(d); 2(e); 2(f)

The exemplified agents of group 2 are identical in every respect to group 1 except that in place of GBP 130 or segments thereof the malaria RBC binding component is provided by GBPH glycophorin binding peptide homologue as referenced herein above especially peptide fragments comprising amino acid residues:

i residue 70 to 427 inclusive

ii residue 109 to 427 inclusive

iii residue 230 to 268 or any other tandem repeat or polymer thereof

of any other fragment or species variation or substitution or deletional or inclusional variant thereof.

EXAMPLE OF AGENTS GROUP 3

The agents of group 3 use the EBA 175 erythrocyte binding antigen 175 for red cell binding.

The EBA 175 erythrocyte binding antigen 175 was cloned and disclosed by B Kim Lee Sim et al Journal Cell Biology, Vol. III, 1990, p1877-1884. See especially Figure 2, p1880 and is incorporated fully herein by reference.

Example 3(a); 3(b); 3(c); 3(d); 3(e); 3(f)

The exemplified agents of group 3 are identical in every respect to group 1 except that in place of GBP 130 or segments thereof the malaria RBC binding compon nt is provid d by EBA 175 erythrocyte binding antigen 175 especially peptide fragments comprising amino acid residues

i residue 20 to 1435 inclusive

of any other fragment or species variation or substitution or deletional or inclusional variant thereof.

EXAMPLE OF AGENTS GROUP 4

The agents of group 4 use the *plasmodium vivax* Duffy Receptor for red cell binding.

The *plasmodium vivax* Duffy Receptor was cloned and disclosed by Xiangdong Fong et al Molecular Biochemical Parasitology, 44 (1991) 125-132. See especially Figure 2, p127 and is incorporated fully herein by reference.

Example 4(a); 4(b); 4(c); 4(d); 4(e); 4(f)

The exemplified agents of group 4 are identical in every respect to group 1 except that in place of GBP 130 or segments thereof the malaria RBC binding component is provided by *plasmodium vivax* Duffy Receptor especially peptide fragments comprising amino acid residues;

i residue 23 to 1051 inclusive

of any other fragment or species variation or substitution or deletional or inclusional variant thereof.

EXAMPLE OF AGENTS GROUP 5

As for group 1 except the malaria parasite peptides are twice represented.

EXAMPLE OF AGENTS GROUP 6

As for group 5 except that 2 or more different malaria parasite peptides are represented.

EXAMPLE OF AGENTS OF GROUP 7

These agents are as for examples 1, 2, 3, 4, 5 and 6 except the malaria parasite derived component is represented all or in part by

i an anti-ideotype Fab fragment

ii an antibody fragment binding to red cells in the same way as the malaria parasite components

EXAMPLES OF MANUFACTURING METHODS

The imaging agents of the present disclosure are fabricated preferably in a stepwise fashion. Many different manufacturing strategies are available for each component any or all of which may be applied in various combinations dictated largely by two factors.

(1) Existing manufacturing facilities for other products within a factory and the cost of alternate strategies.
COST

(2) The minimisation of byproducts which are expensive to produce and eliminate
ie. COST

So many methods of manufacture are now available that economics rather than science dictates the choice.

Initially manufacture will be divided into a number of areas.

(1) The manufacture of polylysin or poly(lysin Zaa) or polys rin or poly(s rineZaa) or poly(Xaa Zaa) where Xaa and Zaa are amino acids.

(2) The manufacture of the merozoite derived peptide components or parts thereof eg. GBP 130.

(3) The fusion of the merozoite peptide fragment of the polylysine or other peptide of (2) above.

Generally it may be economic to produce the merozoite peptide fragment by cloning, and the polylysine by chemical synthesis; coupling to take place thereafter. However, if desired, steps 1, 2 and 3 may be carried out entirely by cloning or synthesis or any mixture of the two.

(4) Coupling of the chelating agent may take place before coupling of polylysine to the merozoite peptide or after, using conventional means.

(5) Coupling of the labelling moiety to the chelating agent will usually but not necessarily be the last step.

In the case where $Tc^{99}m$ or the like is used as the labelling agent then this step will take place just prior to use.

Example I Peptide Manufacture

The art continues to provide new techniques for applying the Merrifield synthesis of peptides on a scaled up basis useful for the large scale manufacture of peptides referred to as scale up of solid phase peptide synthesis, SPPS.

Of the peptide components used to form the agents of the present disclosure the components polylysine $(lys)_n$ or $(lys\ Xaa)_n$ or $(Xaa\ Zaa)_n$ are particularly suitable. Merrifield synthesis of simple poly amino acids such as the above presents few problems.

An inexpensiv technique for manufacturing polylysin involves

(1) The chelating of α amino and α OH groups using Cu^{++} thereby protecting them.

(2) Protection of the side chain NH_2 by benzyl protection.

(3) Removal of Cu^{++} hereby freeing amino and carboxylate groups for peptide binding.

(4) Activation and peptide coupling to be achieved by an inexpensive dycyclohexylcarbonimide DCCD (the one pot reaction).

Coupling of the polylysine to the merozoite peptide fragment may be carried out by conventional means, after first separating polylysine fragment to peptides of desired length by chromatography.

The chemical synthesis of even complex peptides such as an entire merozoite peptide falls well within the scope of the art.

In the interests of brevity a full account of Merrifield synthesis will not be given, however, one is directed to:
The Chemical Synthesis of Peptides, John Jones Clarendon Press, 1991; and to G B Fields and R N Noble 'Sold Phase Synthesis Utilising Fluorenylmethoxycarbonyl amino acids. Int. J Peptide Res 35 1990 p161-214
as useful points of reference and are incorporated fully herein by reference.

The tandemly repeated architecture of some merozoite peptides render their chemical synthesis more economically viable than other large peptides.

Where variations in a peptide sequence are sought ie. substitutional, deletional or insertions of amino acids then it may be very helpful to use Merrifield synthesis for that segment alone.

Newer techniques for multicomponent peptide synthesis permit the simultaneous synthesis of oligo peptide segments in a single run thus reducing time and costs considerably. One is directed to Arpad Fucka et al Int J Peptid Protein Res 37 1991, 487-493 for details disclosures of a method incorporated fully herein by reference.

The present art continues to provide refinements to old techniques and new techniques for peptide synthesis either small quantities for research or larger amounts for industrial purposes. These techniques being familiar to the skilled artisan are disclosed best in standard textbooks and reference texts, accordingly brief reference only will be made to interesting developments which are non limiting.

A recent development involves the use of a new anchoring moiety involving the bonding of (HYCRAM)® (Orpegen GMBh, Czernyring 22, D-6900 Heidelberg FRG) a 4-hydroxy-crotonoyl-amidomethyl grouping bonded to an aminomethyl-polyacrylamide gel via spacer molecules such as B-alanine, or sarcosine or the like. Fmoc-amino acids can be linked to the Hycram® by esterification. Also any Boc-amino acid or any Ddz-amino acid 3, 5-di methoxy phenyl-2-propyl-2-oxycarbanyl-amino acid salt may be bonded to the HYCRAM® anchor.

Synthesis then proceeds using the Boc-/benzyl, the Ddz-/t-butyl or the Fmoc-/t-butyl protocols as usual.

Detachment of the peptide from the HYCRAM® support employs palladium tetra-kis (triphynyl-phosphane) a catalyst in a suitable solvent such as 50% (v/v) dimethylsulphoxide with dimethyl formamide; N-methypyrrolidine, tetrahydrofuran and water. Oxygen tetrahydrofuran must be excluded. Acceptor molecules, morpholine, dimedine or N, N'-di methylbarbiturate may be added to take up the allylic group.

The Ddz-/t-butyl amino acid protections are easier to cleave with 1-5% (v/v) trifluroacetic acid in dichloromethane a proc ss taking 10 to 30 minutes or by means of the more environmental friendly acetic acid or dioxan containing 1% (w/v) HCL gas.

The other useful protocol is the Fmoc-/t-butyl strategy. Cleavage of F moc can b achiev d using 20-50% (v/v) piperidine/dimethyl formamide.

Deprotection can be monitored in both cases photometrically. The activation of Boc-; Fmoc-; or Ddz-amino acid derivatives may employ the inexpensive (Dcc dichlohexylcarbodi-imide. Pre activation using HOBT (N-hydroxybenzotriazole) can be employed to form symmetric anyhdrides of protected amino acids or their esters.

Other activating agents are the Castro Reagent or BOP; Benzotriazole-1-yl-oxy-tris (dimethyl amino) phosphonium hexa flurosphosphate; one is directed to CASTRO B et al (1957) Tetrahedron Lett. 14, 1219; and TBTU the Knorr reagent, Benzotriazole-1-yl-oxy-1, 1, 3, 3-tetramethyluronioum tetrafluoroborate one is directed to Knorr R et al (1989) Tetrahedron Lett. 30, 1927.

Fragment condensation can be achieved using the BOP or the TBTU reagent with HOBT in excess. Protected peptides must also be in excess, however, solvents and excesses can often be recycled.

It will be appreciated that by blocking incomplete fragment condensations shorter by products can be discriminated from the desired polypeptide. Using this system high purity polypeptides can be produced.

Monitoring of production process will usually involve U.V. absorption techniques.

A typical production process involve either the separate synthesis of peptide sequences by their expression in suitable hosts, and their subsequent purification; or chemical synthesis such as on a solid substrate for example by the sequential addition of amino acid residues or peptide fragments which are protected, the protection of the amino acid residues as required and the subsequent reacting of the peptide chains with linking agents before removing the peptide chains from the said solid substrates and the final purification by the various means is such as revers phase chromatography; or any combination of the above.

In the case of some of the exemplary embodiments it may be convenient to manufacture th fusion peptides by means of a fused gene. A fused gene is a g netic s quenc which codes for both components of the hybrid component molecule. One is directed to Murphy United States Patent 4, 675, 382 for a detailed disclosure of the use of fused genes in the manufacture of hybrid peptides having the components MSH or Melanocyte Stimulating Hormone fused to diphtheria A toxins.

Alternatively peptide fragments may be manufactured by DNA cloning and expression in suitable hosts and recovery with subsequent condensation *in vitro*.

Generally cloned sequences useful for the production of fusion peptides will have the transmembrane domain and the cytoplasmic domain sequence removed.

For a useful general description of DNA cloning and molecular hybridization technology, one is directed to Maniatis et al Mollecular cloning, A Laboratory Manual, Cold Spring Harbour Laboratory (See Second Edition 1989); and to Horvath et al, An Automated DNA synthesizer employing Deoxynucleotide 3 Phosphoramidites, Methods in Enzymology 154: 313-326, 1987.

DNA may be made by the chemical synthesis of DNA polymer fragments using phosphotriester, phosphite or phosphoramidite chemistry. For a description of sold phase techniques one is directed to Chemical and Enzymatic Synthesis of Gene Fragments - A Laboratory manual ed H G Gassen and L Lang, Verlag Chemiee, Weinheim 1982; and Gait M J et al Nucleic Acids Research 1982, 10, 6243; Spoat B S et al Tetrahedron Letters, 1980, 21, 719; Matteuci M D et al J. American Chemical Society, 1981, 103, 3185, Adams S P et al J of American Chemical Society 1983, 195, 661; Sinha N D et al Nucleic Acids Research, 1984, 12, 4539 and Matthes H W D et al Embo. Journal, 1984, 3, 801, whose teachings are incorporated herein fully by reference.

Reverse transcriptase techniques may also be used to generate a complimentary c DNA strand by means of the revers transcription of malaria parasit d rived mRNA. Kits are availabl for this purpose.

The DNA fragments may be ligated by either blunt-ended or staggered-ended termini after using restriction enzymes; digestion; filling in as required; and treatment with alkali and phosphatase for protection and subsequent ligation with suitable ligases.

Appropriate leader sequences may be chosen from the many available.

The cloning of the DNA sequences of the hybrid peptides of this invention may take place in prokaryotes such as *E.Coli* for example, K12 strain or *E.Coli* B by way of non limiting examples or by means of the polymerase chain reaction.

Subsequent expression of the hybrid peptides may take place in any host cell, including mammalian host cells. Other useful cells are fungi, yeasts, insects and prokaryotes. Signals suited to the chosen host cell are chosen as appropriate, in the case of prokaryotes one can choose from a large group including alkaline phosphatase, pemallinase and the like.

Where prokaryotes such as *E.Coli* for example, are used to express the hybrid peptides, then they are transformed by an expression vector usually a plasmid such as PBR322 into which the DNA encoding the fusion peptide or fragment has been ligated such a plasmid will also feature suitable marker sequences, promoters, and Shine-Dalgarno sequences may be chosen as appropriate.

A prokaryote host such as *E.Coli* may be transformed by treatment using a solution of $CaCl_2$ as described by Cohen et al PNAS 1973, 69, 2110 or by treatment with a solution comprising a mixture of RbCl, $MnCl_2$, potassium acetate and glycerol and then subsequently with 3 - [N-morpholino] - propene-sulphonic acid and, RbCl and glycerol.

One is directed to "DNA Cloning" Vol II D M Glover ed, IRL press Ltd 1985 for a description of transforming techniques.

Where insect cells such as *Lepidoptera* cells are the chosen expression host a suitable vector would be Baculovirus. Such a system would contain the targ t peptid ncoding sequence linked to a baculovirus promoter within a shuttle vector with sufficient

baculovirus DNA flanking the target peptide encoding sequence to permit recombination. One is directed to Summers et al, TAES Bull (Texas Agricultural Experimental Station Bulletin) NR 1555 May 1987. One is also directed to Smithklein (WO/US/89/05550). Insect larvae can also be used to produce transformed insect cells, particularly *Heliothis virescens* caterpillars and one is directed to PCT/WO/88/0200030 Miller et al. Other useful insects are *Drosophila melanogaster*, and the like.

Where plant cells are the chosen host expression cells, the cowpea plant provides a suitable expression system. One is directed to the system developed by the Agriculture Genetics Company of Cambridge, UK, employing techniques involving the use of the cowpea mosiac virus (CPMV). A general protocol for the cloning of foreign genes in plants (Tomatoe) and the like may be obtained by consulting HORSCH R B et al Science 227, 1229-31.

Alternatively where the chosen host is a yeast such as *Saccharomyces cerevisiae* the plasmid YRp7 as the expression vector may be used. One is directed to Stinchcomb et al Nature 282, 39, (1979), Kingsman et al, G7; 141 (1979); Tschemper et al, Gene 10; 175 (1980).

A wide choice of promoters is available for use in yeast cell expression systems and include by way on non limiting examples 3-phosphoglycerate kinase and one is directed to Hitzman et al J. Biol. Chem 255 2073 (1980); also enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triose phosphate isomerase, phosphoglucose isomerase and glucokinase being glycolytic enzymes and one is directed to Hess et al J. Adv. Enzyme Reg. 7, 149 (1968) and to Holland Biochemistry 17, 4900;

Other promoters suitable for yeast expression systems include the promoter regions for alcohol dehydrogenase 2, 100 cytocrome C, acid phosphatase, also mettallothioneins, glyceraldehyde-3-phosphate dehydrogenase and others one is directed to Hitzman R et al European patent Publication No. 73, 657A.

Where mammalian cells are th chos n hosts for expression, these cells may be grown *in vitro* in tissue culture or suitable bioreactors or *in vivo* in animals.

Vectors useful for mammalian cells host systems involve the use of DNA derived from animal viruses such as SV40 virus; retroviruses such as RSV, MMTV, MOMLV, baculovirus, Vaccinovirus, Adenovirus, polyoma or bovine papilloma virus.

Promoters suitable for mammalian cells systems may be chosen from the many available. One is directed to Friers et al Nature 273; 113 (1987) and Greenway P J et al Gene 18, 353-360 (1982) and Okayamah Mol Cell Biol. 3, 280 1 1983, by way of example.

Additionally suitable enhancers may be chosen from the many available. One is directed to Laimins L et al PNAS 78, 993 (1981) and Lusky M L et al Mol. Cell Bio. 3, 1108 (1983) and Banerji J L et al Cel. 33, 729 (1983) and Osbourne T F et al Mol. Cell. Bio. 4, 1293 (1984).

For a description of some available selection techniques one is directed to Southern et al J. Molec. Appl. Genet. 1, 327 (1982) and to Mulligan et al Science 209; 1422 (1980) and to Sugden et al Mol. Cell. Biol. 5, 410-413 (1985).

Some techniques useful for the introduction of the expression vector into the host cell involve protoplast fusion, calcium phosphate precipitation, electroporation, and other techniques.

Mammalian host cells suitable for the expression of fragments of or in some cases the entire fusion peptides may now be chosen from the large number now available such as VERO or CHO-K1, a myeloma cell line.

Other suitable eukaryotic host cells include COS cells, human embryonic kidney cells, mouse plasmacytoma cells, mouse sertoli cells, baby hamster kidney cells (BHK cells), Chines hamster ovary cells - DKFR (CHO), monkey kidney c lls, African green monkey kidney c lls, human cervical carcinoma cells (HELA cells), canine kidney cells, Buffalo

rat liver cells, human lung cells, human liver cells and mouse mammary tumour cells, by way of non limiting xamples.

It will be appreciated that the chosen host cells will preferably express the minimum levels proteases within their cytoplasm. It will also be appreciated that amino acid sequence variations of the peptide sequences involved may be insertions, substitutional or deletional variations involving single amino acid residues or peptide fragments.

The purpose for such variations may be to increase the affinity of the components, to improve stability, to reduce the cost preparation or to increase the half life, or to lessen the severity of side effects such as atopic reactions. The final form of the agents of the present disclosures may involve any combination of substitutions, deletion or insertion of amino acid sequences, provided the binding ability of the epitopes or peptide sequence are retained.

It will be appreciated that also included in the present disclosure are glycosylation variations ie. variants completely unglycolated, variants having glycosylated amino acids other than those glycosylated in the natural peptides or variants having a greater number of amino acid residues glycosylated or fewer glycosylated residues or residues glycolated by oligosaccharides other than those oligosaccharides usually associated with the said sequences.

Following their expression by the host cell, the peptides are recovered and purified by means known to the ordinary skilled artisan.

Such methods may include acid extraction, ethanol precipitation using ammonium sulphate, anion or cation exchange chromatography, phosphocellulose chromatography, immuno-affinity chromatography, hydroxyappetite chromatography and reverse phase chromatography.

Generally purification and processing involves four stages:

(1) extraction of peptides from host cell

(2) initial purification

(3) final purification

(4) production polishing

Extraction may be accomplished using sonication techniques or solid shear techniques - on a small scale.

Lysozyme based techniques are expensive. More usually in the case of bacterial hosts homogenizers or liquid shear techniques are employed.

Other useful techniques involve osmotic shock, freezing and thawing or alkali homogenisation.

In the case of where a product is expressed as an inclusion body cell paste may be solubilised by solvents such as 8M-guanidinium.

Centrifugation provides the removal of cellular debris, and continuous flow centrifuges are preferred for large scale operations. As an alternative to centrifugation cross flow filtration using flat to tubular membranes and high shear forces may provide a useful alternative to centrifugation.

Initial purification involves mainly the removal of excess water and product concentration.

Precipitation using ammonium sulphate, organic solvents, polyethylene glycol or other polymers can be used to accomplish this step; with the addition of some variety of chromatography usually absorptive chromatography techniques employing ion exchange, hydrophobic or bioaffinity interactions; followed by washing and desorption; chromatography is not restricted to columns but may take place in membranes or even in spirally wound cartridges.

In cas s wher peptide fragments have been expressed as inclusion bodi s an additional step of 'refolding' is required. Because of low yields after refolding inclusion body production is often uneconomical. However two approaches are practised to refold

peptides into the natural or desired three dimensional state the empirical approach and the rationalist approach.

In the empirical approach multiple solvents are applied and an optimum strategy is determined using phase diagrams as disclosed by Ahmed and Biglow 1979 J. Mol. Biol. 131. 6097.

The rationalist approach seeks to produce conditions favouring the native state while at the same time keeping intermediates in solution.

A problem may arise in connection with disulphide bridges which may form in non-native configurations. A way around this problem is to oxidize disulphides under denaturing conditions, using gel filtration remove covalent aggregates and thereafter dilute the product in a non denaturing buffer. The peptide which collapses into an amorphous tangle often rearranges itself into the native form.

Highly resolving chromatography is the preferred technique for final purification. For large scale applications columns are preferred and techniques such as gel filtration, ion exchange, hydrophobic interaction or affinity chromatography may be used alone or in combination as dictated by economies of scale.

Gel filtration is best suited to small batch volumes and suffers from the disadvantage of slow speed.

Ion exchange chromatography techniques are very useful in early purification stages and can deal with large volumes at great speed, producing yields of high resolution.

Hydrophobic interaction chromatography provides both high resolution and high speed even at large batch volumes.

Bio affinity chromatography produces the highest resolution, at high speed, but batch size may require curtailment. This techniqu is an ideal late stage technique.

The increasing availability of monoclonal antibodies at lower prices has led to greater use of bio affinity chromatographic techniques.

A wide choice of chromatographic matrices is now available on the market suitable for large scale use.

Particle size is decreasing from the 90μm of traditional gels to approximately 40μm for newer gels such as Sepharose HR®, Sephacryl HR®, Superdex® (Pharmacia - LKB) or Fractogel® (Toso-Haas).

Other polymeric particles are Superose® (Pharmacia - LKB) or the TSKPW® varieties manufactured by (Toso Haas, Philadelphia USA), providing very low particle size.

It will be appreciated that the process of chromatography involves the choice and development of a strategy containing one or more steps ie. high resolution single step or a multistep procedure the final choice to be determined by

(1) the chosen peptide production process which determines the form of the starting material to be purified

(2) cost

Usually, the first chromatography column will involve large diameter packings circa 100μm, which are often chosen so that they can be resanitized by sodium hydroxide to reduce costs. Low resolution steps to be followed by higher resolution steps until the desired product purity is obtained.

A typical strategy might involve

Step On Hydrophobic interaction chromatography at low ionic strength. Purpose to absorb proteinases. Target peptide not absorbed and collected. Alternatively use proteinase inhibitors.

Step Two — Re-run through h.l.c. column adding salts to bind the target peptid to the h.l.c. column. The objective is volume reducti n. Alternatively employ ultra filtration as described. Use a step gradient to elute the target peptide.

Step Three — A step gradient to a low ionic strength buffer will remove remaining salts. Alternatively employ polyethlyleneimine precipitation, centrifugation and diafiltration.

In the case of therapeutic peptides such as the agents of this disclosure where administration to a human is considered then the step of produce polishing is vital.

Product polishing involves the removal of polymers of the product and other pyrogens.

Techniques for product polishing involve additional gel filtration with or without a buffer exchange step; treatment with alhydrogel (aluminium hydroxide) or treatment with specific lecithins or anion exchanges.

EXAMPLE 2

THE COUPLING OF PEPTIDES TO RADIOACTIVE OR TARGETED MOIETY

In the case of labelling of peptides with technetium the half life of $Tc^{99}m$ being 6 hours requires that radiolabelling take place prior to administration of the imaging agent.

$Tc^{99}m$ is easily obtained in pharmacologically acceptable form from a conventional ^{99} Mo-^{99} mTc generator.

A direct method of coupling disclosed by Rhodes, US patent 4,305,922, may be used but is somewhat cumbersome.

Having reduced pertechnetate to technetium IV and allowed the technetium IV to bind to Sephadex® column the peptid to be labelled is applied to the column.

In this situation t chnetium IV pref rentially binds to th prot in to b labell d and quits th Sephadex®. Th effici ncy of the process may be improved by "pre-tinning" the protein to be labelled using stannous chloride one is directed to US patent 4,424,200 incorporated herein by reference.

More preferably chelating agents such as DTPA or the others mentioned herein before are bound first to the protein and the radionucleide bound to the chelating agent. Khaw et al Science 209: 295-297 (1980) discloses peptides labelled with Indium III using DTPA as a chelator; one is also directed to Krejcarek et al Biochem Biophys Res Commun 77: 581-585 (1977) and to Childs R L et al J. Nucl. Med. 26: 293 (1985) and still further directed to Fritzberg et al J. Nucl. Med 27: 957 (1986) and also to EP applic 86100360.6 for disclosure relating to the coupling of targeted moieties to peptides via chelating agents.

One is also directed to WO88/07382 for a description of the use of polyhydroxydicarboxylic acids as chelating agents useful for coupling metals to peptides especially saccharic acid, disclosed and incorporated fully herein by reference.

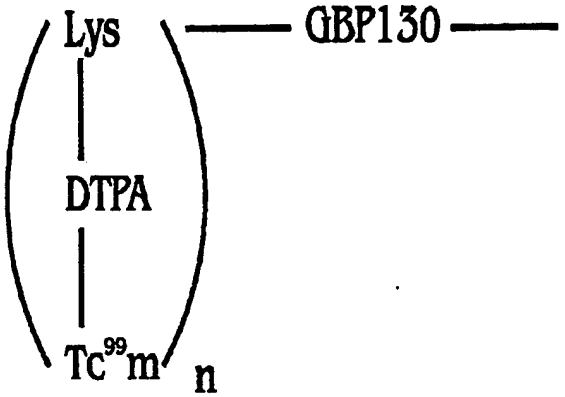
More specifically the purified peptides to be bound to a chelator such as DTPA is conveniently immobilised as resin and in the presence of di isopropylethlyamine reacted with an appropriate quantity of DTPA bis anhydride. On completion of the reaction the peptide resin is washed with DMF dimethylformamide, water, dichloromethane and ether.

The dried resin may be treated with mixtures of Tri fluoroacetic acid TFA to release the peptide. After precipitation of peptide by evaporation of TFA the peptide may be purified by conventional means using reverse phase chromatography. The peptide chelate is now ready for reaction with the radionucleotide or labelling entity.

This technique has been successfully employed to manufacture DTPA-$(MSH)_{bis}$ a radiolabelled agent directed against melanoma and one is directed to WO 90/03798 disclosed and incorporated fully herein by reference.

Example 2: Th Clinical T sting of Radiolabelled Agents

In the cas of radiolabelled ag nts such as examples radiolabelled by $Tc^{99}m$ for example



The agents may be tested in dogs by first labelling human red cells with the agent. Dogs capable of tolerating an injection of human red cells are anaesthetized. The heart is mobilised and an infarct is produced by ligating or diathermying a branch of the left anterior descending coronary artery. The agents are injected as a bolus intravenously.

$Tc^{99}m$ images are obtained using a gamma camera set to a KeV140 photopeak and 20% window.

Example 3: The clinical testing of the present agents for use in computerised tomography.

Anaesthetized dogs may be used to assess the GBP 130 agents either labelled by Gd or Yt or iodinated side chains as described in the examples.

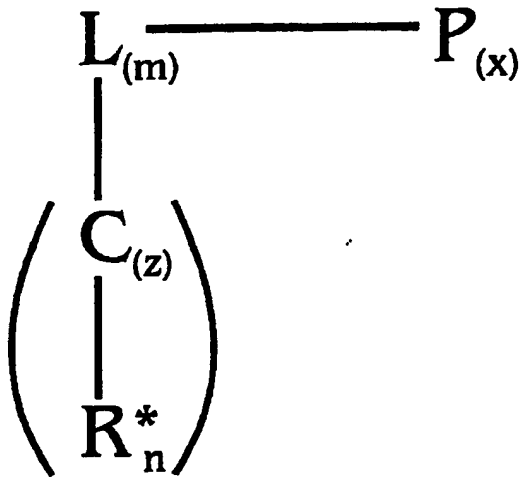
Where Gadolinium or Dysprosium agents are used for CT imaging then useful enhancement is observed using 1ml/kg of 0.5 molar Gd agent or 0.5 molar Dy 1ml/kg.

It must be emphasised that calibration of CT machines is essential arising from the fact that image quality varies from CT machine to machine despite use of similar concentrations of imaging agents.

CLAIMS

What is claimed is:

1. An imaging agent having the general formula



- Where P is a peptide derived from the malaria parasite and is capable of binding to a Red Cell or a substitutional deletional or insertional variation or such a peptide or a strain or species variation of such a peptide;

- Where L represents an amino acid or poly amino acid linker $(Xaa)_m$ or $(XaaZaa)_m$ or any non-malarial derived peptide fragment;

- Where 'x' is a natural number;

- Where 'm' is a natural number;

- Where C is a chelator or chelating agent capable of binding both to a side chain of an amino acid and to a labelling entity.

- Where R* is a labelling entity either element or molecule;

- Where 'n' is a natural number;

- and wher 'z' is a natural number.

2. An imaging agent according to claim 1 where P represents the malaria derived peptide component and is all or part of GBP 130 (glycophorin binding peptide 130) especially a peptide fragment comprising

amino acid residues	201	to	774 inclusive
	202	to	774 inclusive
or	370	to	426 inclusive

or other single tandem repeat or polymer thereof or polymer of a fragment thereof or substitutional deletional or insertional variation or strain or species variation thereof.

3. An imaging agent according to claim 1 where P represents the malaria derived peptide component and is all or part of GBPH, glycophorin binding peptide homologue especially a peptide fragment comprising

amino acid residues	70	to	427 inclusive
or	109	to	427 inclusive
or	230	to	268 inclusive

or other single tandem repeat or polymer thereof or polymer of a fragment thereof or substitutional deletional or insertional variation or strain or species variation thereof.

4. An imaging agent according to claim 1 where P represents the malaria derived peptide component and is all or part of the EBA 175 molecule (Erythrocyte Binding Antigen) especially a peptide fragment all or part or derived from

amino acid residues	20	to	1435 inclusive

or a smaller fragment thereof or polymer thereof or polymer of a fragment thereof or substitutional deletional or insertional variation or strain or sp cies variation ther of.

5. An imaging agent according to claim 1 where P represents the malaria deriv d peptide component and is all or part of the Plasmodium Vivax Duffy Receptor molecule especially a peptide fragment derived from or comprising

amino acid residues 23 to 1051 inclusive

or other fragment thereof or polymer thereof or polymer of a fragment thereof or substitutional deletional or insertional variation or strain or species variation thereof.

6. An imaging agent according to Claim 1, 2, 3, 4, 5 where the malaria parasite peptide fragment is represented twice.

7. An imaging agent according to Claim 1, 2, 3, 4, 5, 6, where the malaria parasite peptide fragment(s) are two or more different malaria parasite derived peptides.

8. An imaging agent according to Claim 1, 2, 3, 4, 5, 6, 7 where the malaria parasite peptide fragment(s) are represented by anti ideotypes or Fab antibody component capable of binding to a Red Cell surface in a similar way as a malaria parasite peptide.

9. The use of a malaria parasite peptide fragment, GBP 130 (glycophorin binding peptide 130) especially a peptide fragment comprising

amino acid residues		201	to	774 inclusive
		202	to	774 inclusive
	or	370	to	426 inclusive

or other single tandem repeat or polymer of a fragment thereof or substitutional or deletional or insertional or strain or species variation thereof, labelled by a labelling element or group especially $Tc^{99}m$, I^{132}; I^{131}; In^{111}; Ru^{97}; Cu^{67}; Ga^{67}; Ga^{68}; Au^{145}; Gd^{157}; Mn^{55}; Dy^{162}; Cr^{52}; Fr^{56} or the luminescent l ment Eu^{152}. Chemically linked by a chelating agent especially DTPA; DTPABMA; DOTA; HP-

DO3A; EDTA; EGRA; TTHA; HBED; directly to the parasite derived peptide via side chain condensation with an amino acid especially serine, or indirectly via polymers of serine, lysine or any intermediate peptide, poly(Xaa)$_m$; poly(XaaZaa)$_m$; to obtain a medicine for intended therapeutic use as a diagnostic imaging agent to image blood flow or vascular function or pathology.

10. The use of a malaria parasite peptide fragment GBPH, glycophorin binding peptide homologue especially a peptide fragment comprising

amino acid residues		70	to	427 inclusive
	or	109	to	427 inclusive
	or	230	to	268 inclusive

or other single tandem repeat or polymer of a fragment thereof or substitutional or deletional or insertional or strain or species variation thereof, labelled by a labelling element or group especially $Tc^{99}m$, I^{132}; I^{131}; In^{111}; Ru^{97}; Cu^{67}; Ga^{67}; Ga^{68}; Au^{145}; Gd^{157}; Mn^{55}; Dy^{162}; Cr^{52}; Fr^{56} or the luminescent element Eu^{152}. Chemically linked by a chelating agent especially DTPA; DTPABMA; DOTA; HP-DO3A; EDTA; EGRA; TTHA; HBED; directly to the parasite derived peptide via side chain condensation with an amino acid especially serine, or indirectly via polymers of serine, lysine or any intermediate peptide, poly(Xaa)$_m$; poly(XaaZaa)$_m$; to obtain a medicine for intended therapeutic use as a diagnostic imaging agent to image blood flow or vascular function or pathology.

11. The use of a malaria parasite peptide fragment, the EBA 175 molecule (Erythrocyte Binding Antigen) especially a peptide fragment all or part or derived from

amino acid residues	20	to	1435 inclusive

or a smaller fragment thereof of a fragment thereof or polymer of a fragment thereof or substitutional or deletional or insertional or strain or species variation thereof, labelled by a labelling element or group especially $Tc^{99}m$, I^{132}; I^{131};

In^{111}; Ru ^{97}; Cu^{67}; Ga^{67}; Ga^{68}; Au^{145}; Gd^{157}; Mn^{55}; Dy^{162}; Cr^{52}; Fr^{56} or the luminescent element Eu^{152}. Chemically linked by a chelating agent especially DTPA; DTPABMA; DOTA; HP-DO3A; EDTA; EGRA; TTHA; HBED; directly to the parasite derived peptide via side chain condensation with an amino acid especially serine, or indirectly via polymers of serine, lysine or any intermediate peptide, $poly(Xaa)_m$; $poly(XaaZaa)_m$; to obtain a medicine for intended therapeutic use as a diagnostic imaging agent to image blood flow or vascular function or pathology.

12. The use of a malaria parasite peptide fragment, the Plasmodium Vivax Duffy Receptor molecule especially a peptide fragment derived from or comprising

amino acid residues 23 to 1051 inclusive

or other fragment thereof or polymer of a fragment thereof or substitutional or deletional or insertional or strain or species variation thereof, labelled by a labelling element or group especially $Tc^{99}m$, I^{132}; I^{131}; In^{111}; Ru^{97}; Cu^{67}; Ga^{67}; Ga^{68}; Au^{145}; Gd^{157}; Mn^{55}; Dy^{162}; Cr^{52}; Fr^{56} or the luminescent element Eu^{152}. Chemically linked by a chelating agent especially DTPA; DTPABMA; DOTA; HP-DO3A; EDTA; EGRA; TTHA; HBED; directly to the parasite derived peptide via side chain condensation with an amino acid especially serine, or indirectly via polymers of serine, lysine or any intermediate peptide, $poly(Xaa)_m$; $poly(XaaZaa)_m$; to obtain a medicine for intended therapeutic use as a diagnostic imaging agent to image blood flow or vascular function or pathology.

13. The use of a malaria parasite peptide fragment, where the malaria parasite peptide fragment(s) are represented by anti ideotypes or Fab antibody component capable of binding to a Red Cell surface in a similar way as a malaria parasite peptide or polymer of a fragment thereof or substitutional or deletional or insertional or strain or species variation thereof, labelled by a labelling element or group especially $Tc^{99}m$, I^{132}; I^{131}; In^{111}; Ru^{97}; Cu^{67}; Ga^{67}; Ga^{68}; Au^{145}; Gd^{157}; Mn^{55}; Dy^{162}; Cr^{52}; Fr^{56} or the luminescent element Eu^{152}. Chemically linked by a chelating agent especially DTPA; DTPABMA; DOTA; HP-DO3A; EDTA; EGRA; TTHA; HBED; directly to the parasite derived peptid anti ideotype or Fab via side

chain condensation with an amino acid especially serine, or indirectly via polymers of serine, lysine or any intermediate peptide, poly(Xaa)$_m$; poly(XaaZaa)$_m$; to obtain a medicine for intended therapeutic use as a diagnostic imaging agent to image blood flow or vascular function or pathology.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number
GB 9313965.7

Relevant Technical fields

(i) UK Cl (Edition L) C3H (HHX2)

(ii) Int Cl (Edition 5) A61K 49/00, 49/02

Search Examiner
C SHERRINGTON

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI, CLAIMS, DIALOG/BIOTECH

Date of Search
4 OCTOBER 1993

Documents considered relevant following a search in respect of claims 1-13

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
	NO DOCUMENTS FOUND	

Category	Identity of document and relevant passages -60-	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if c mbined with one or more other documents of the same category.

A: Document indicating technol gical background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present applicati n.

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